SUBSTITUTE SHEET (RULE 26)

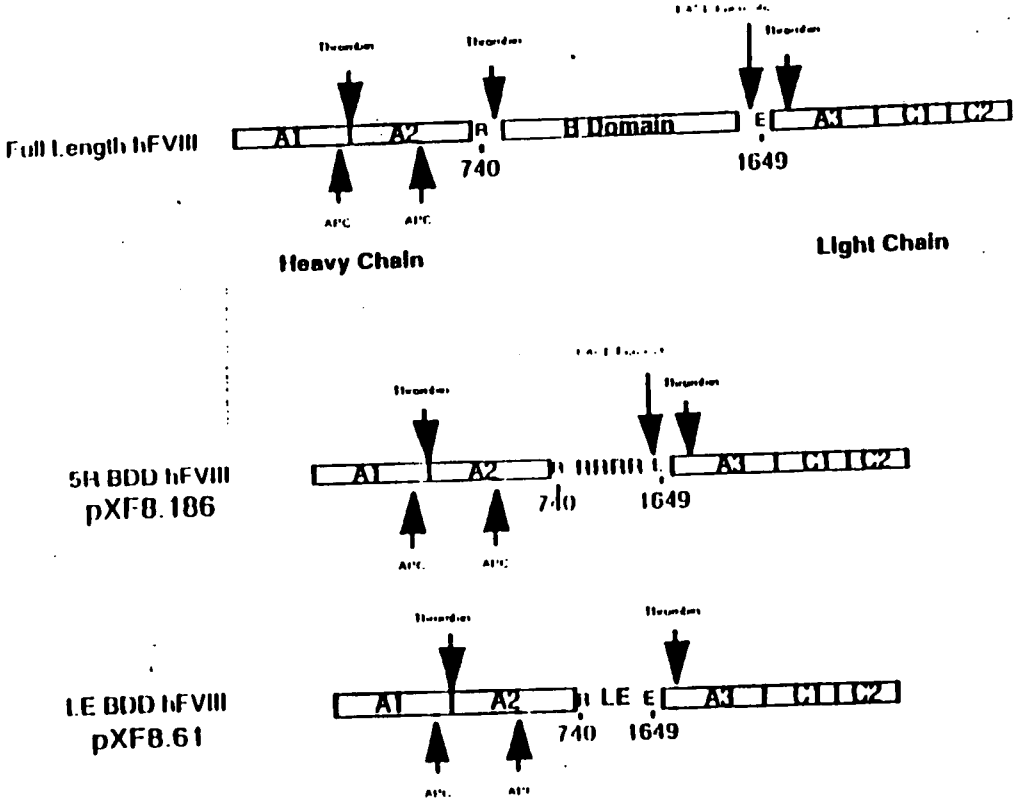
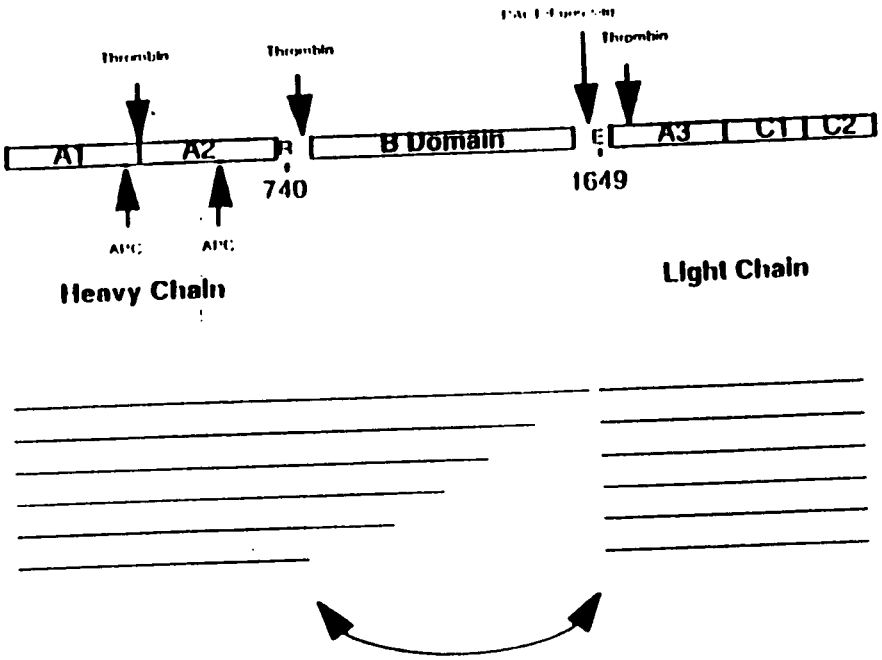


FIG. 1



Heterogeneity of hFVIII is due to proteolysis within the B-domain

FIG. 2

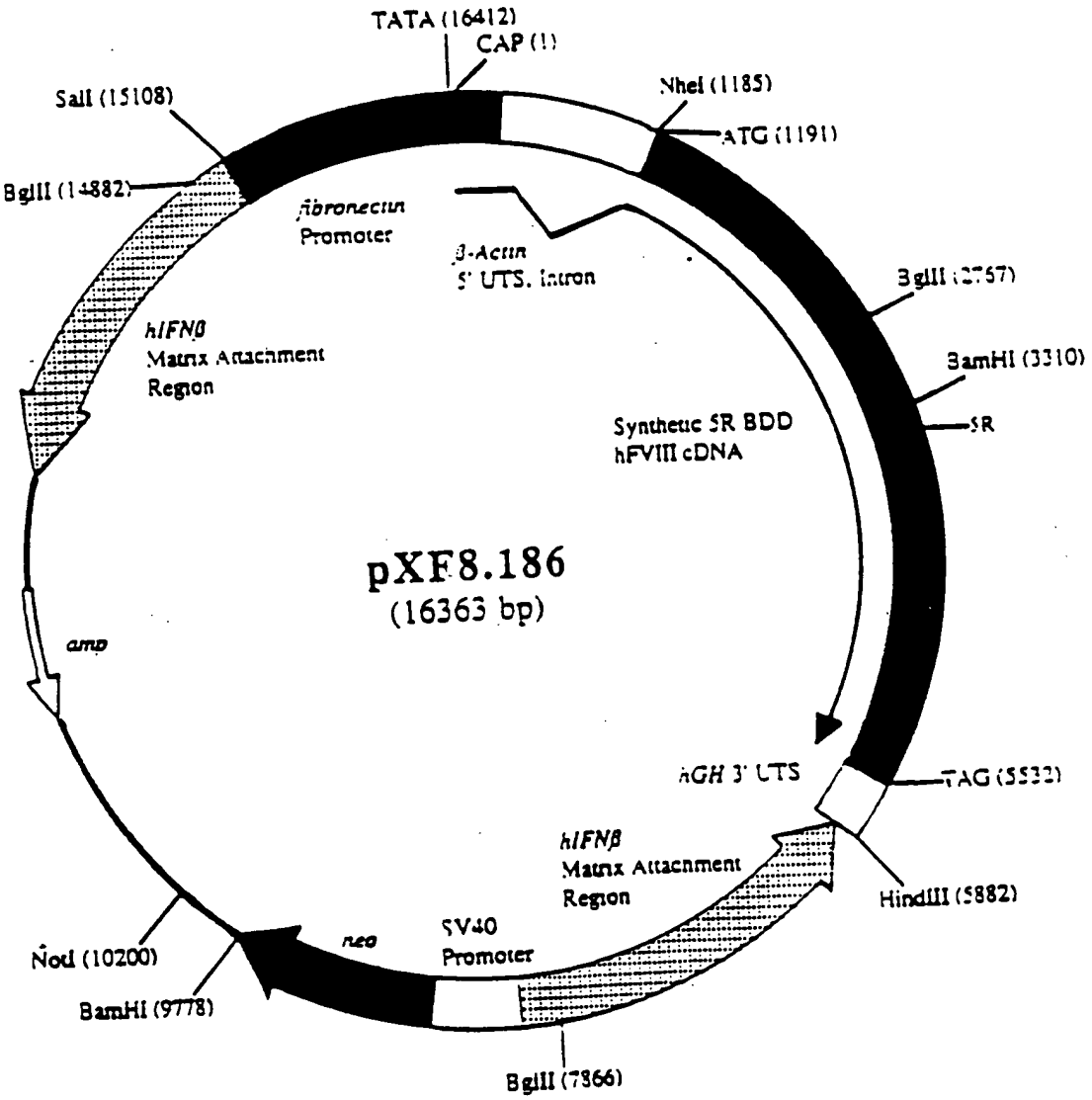

TATA (16412)
CAP (1)
NheI (1185)
ATG (1191)
SalI (15108)
BglII (14882)
fibronectin
Promoter
β-Actin
5' UTS. Intron
BglII (2767)
BamHI (3310)
SR
hIFNβ
Matrix Attachment
Region
Synthetic SR BDD
hFVIII cDNA
pXF8.186
(16363 bp)
amp
hGH 3' UTS
TAG (5532)
hIFNβ
Matrix Attachment
Region
HindIII (5882)
SV40
Promoter
neo
NotI (10200)
BamHI (9778)
BglII (7866)

FIG. 3

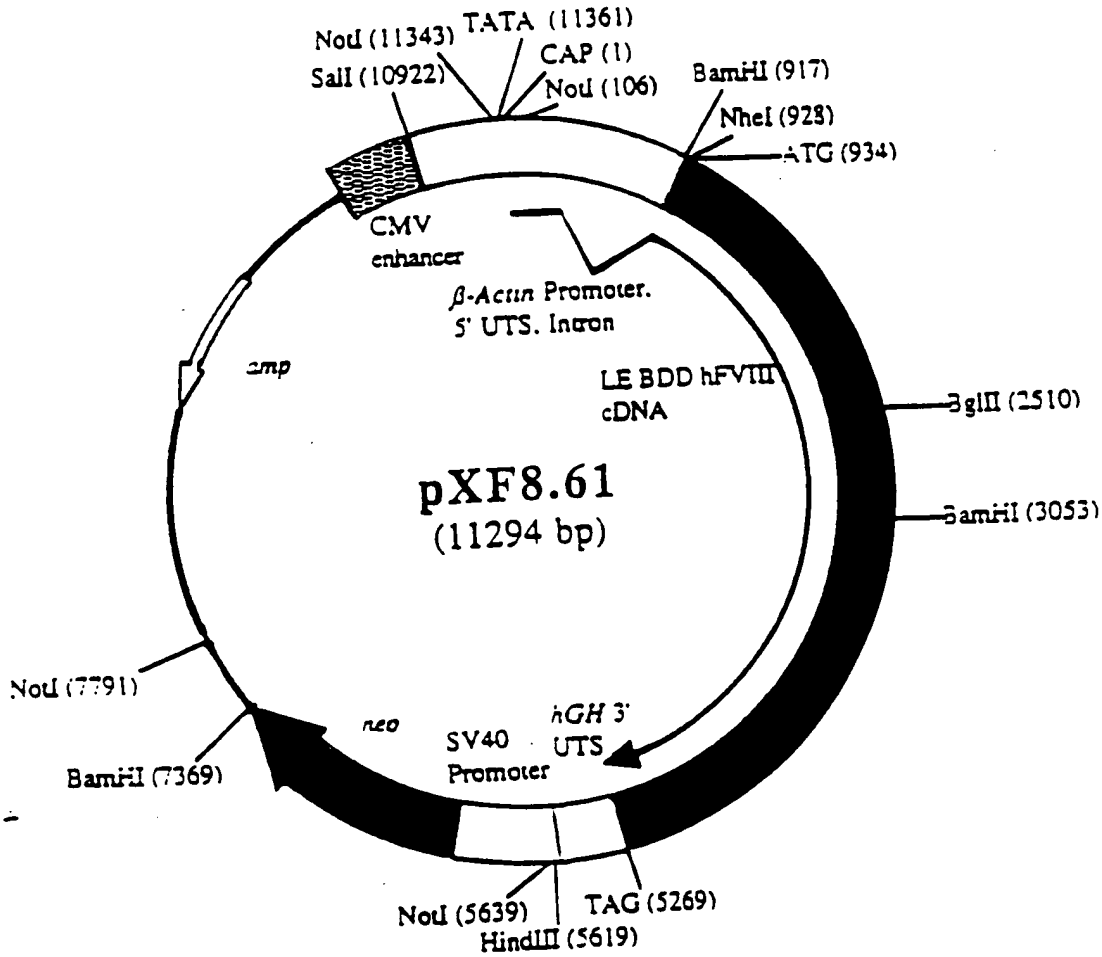

NotI (11343)
TATA (11361)
CAP (1)
SalI (10922)
NotI (106)
BamHI (917)
NheI (928)
ATG (934)
CMV
enhancer
β-Actin Promoter.
5' UTS. Intron
LE BDD hFVIII
cDNA
BglII (2510)
BamHI (3053)
pXF8.61
(11294 bp)
amp
NotI (7791)
neo
hGH 3'
SV40
UTS
Promoter
BamHI (7369)
NotI (5639)
TAG (5269)
HindIII (5619)

FIG. 4

Fragment A

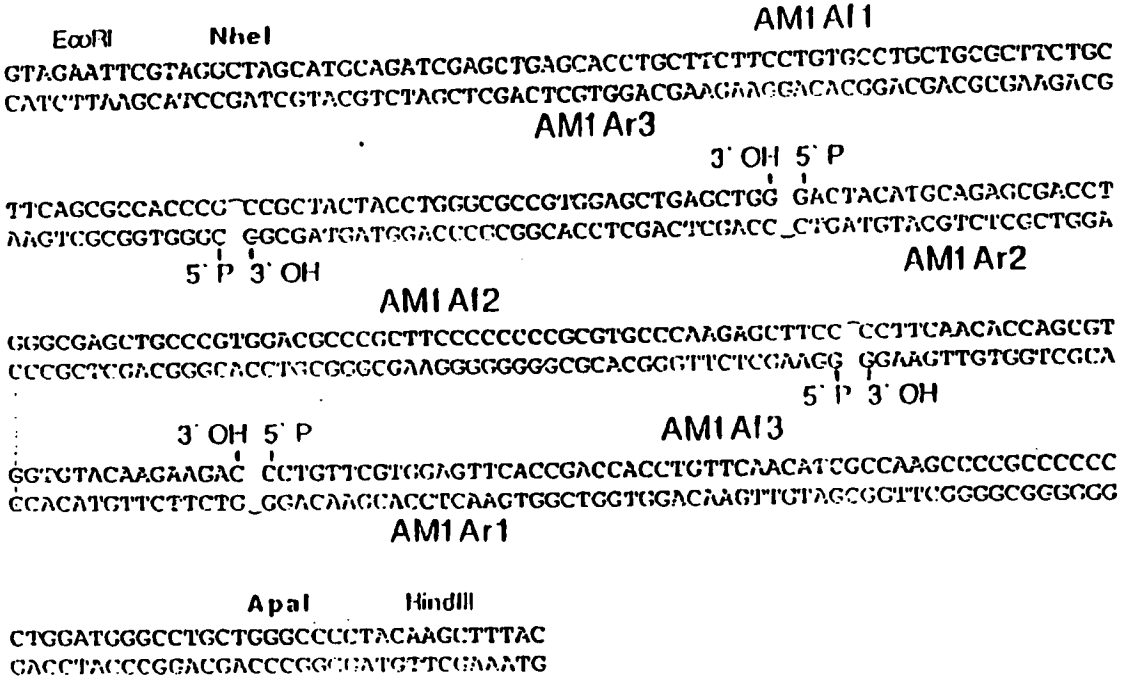


FIG. 5 (1 of 14)

Fragment B

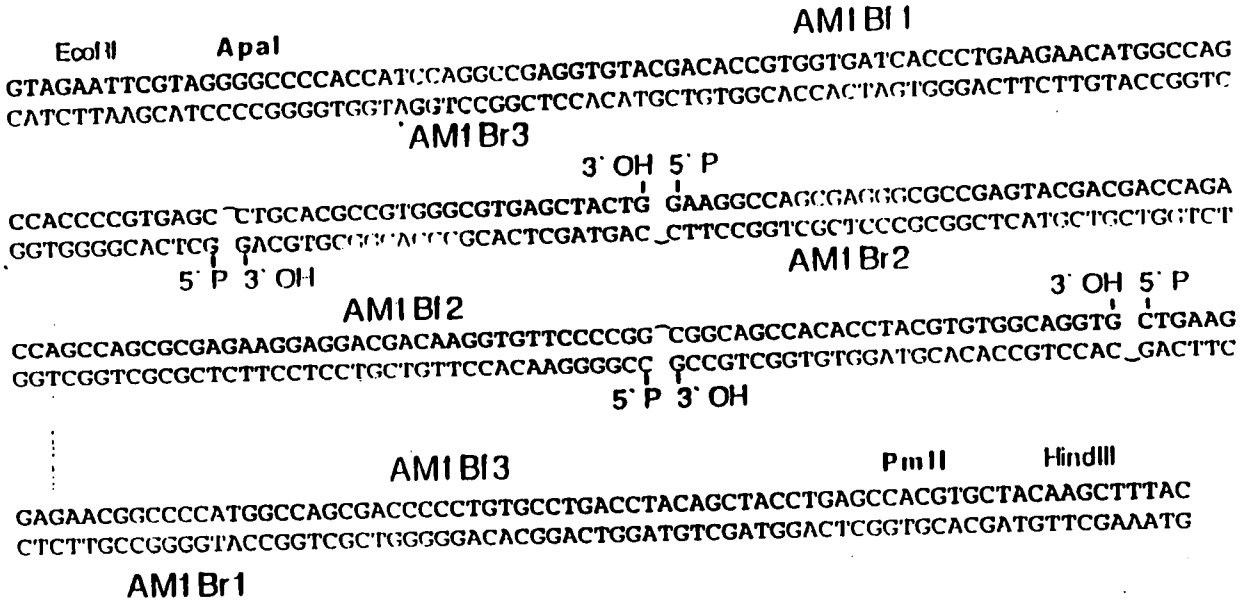


FIG. 5 (2 of 14)

Fragment C

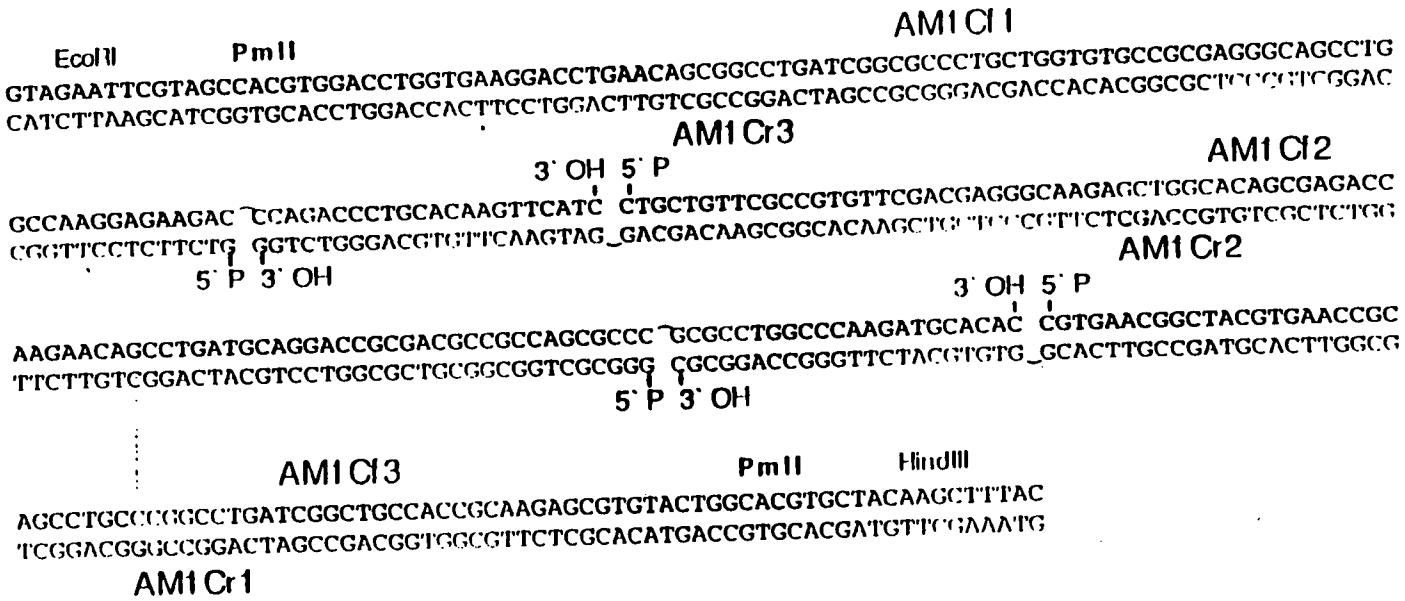


FIG. 5 (3 of 14)

Fragment D

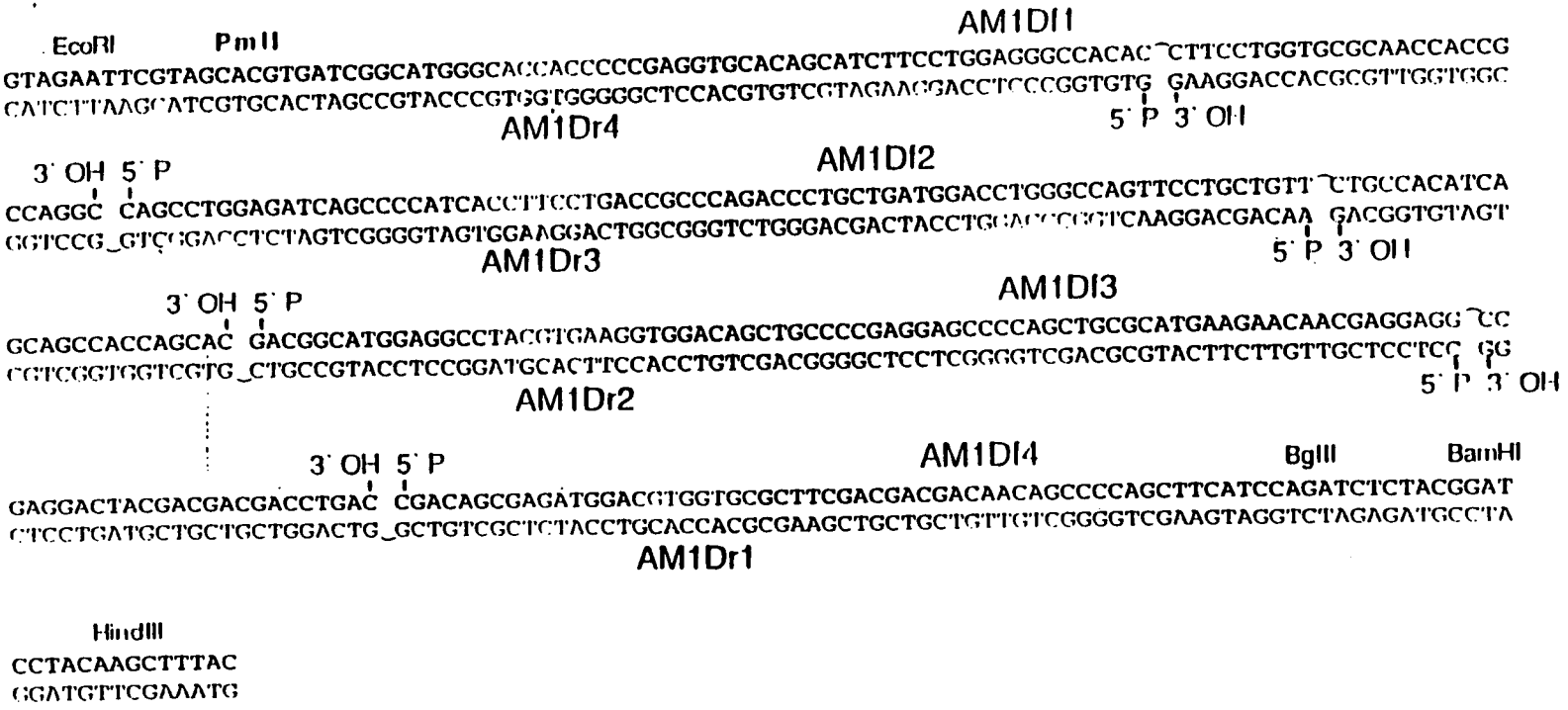


FIG. 5 (4 of 14)

Fragment E

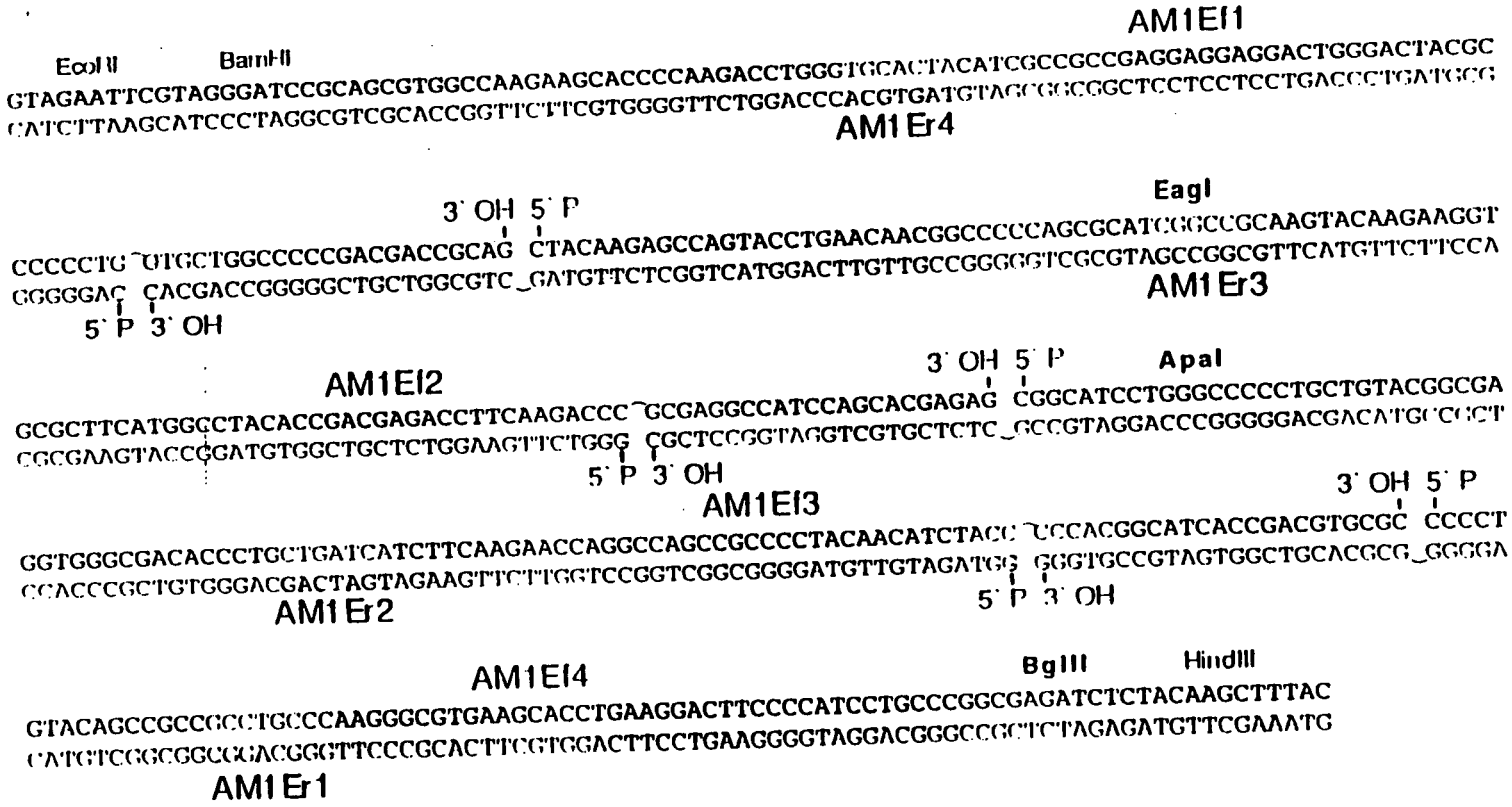


FIG. 5 (5 of 14)

Fragment F

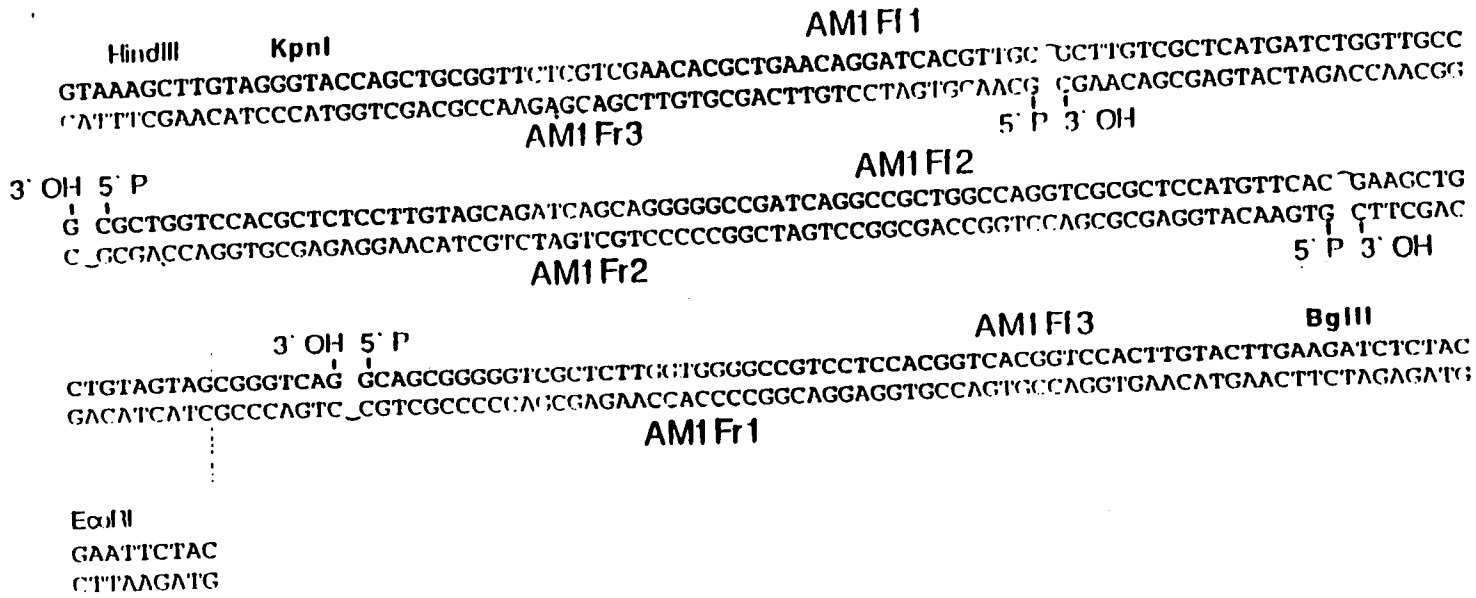


FIG. 5 (6 of 14)

Fragment G

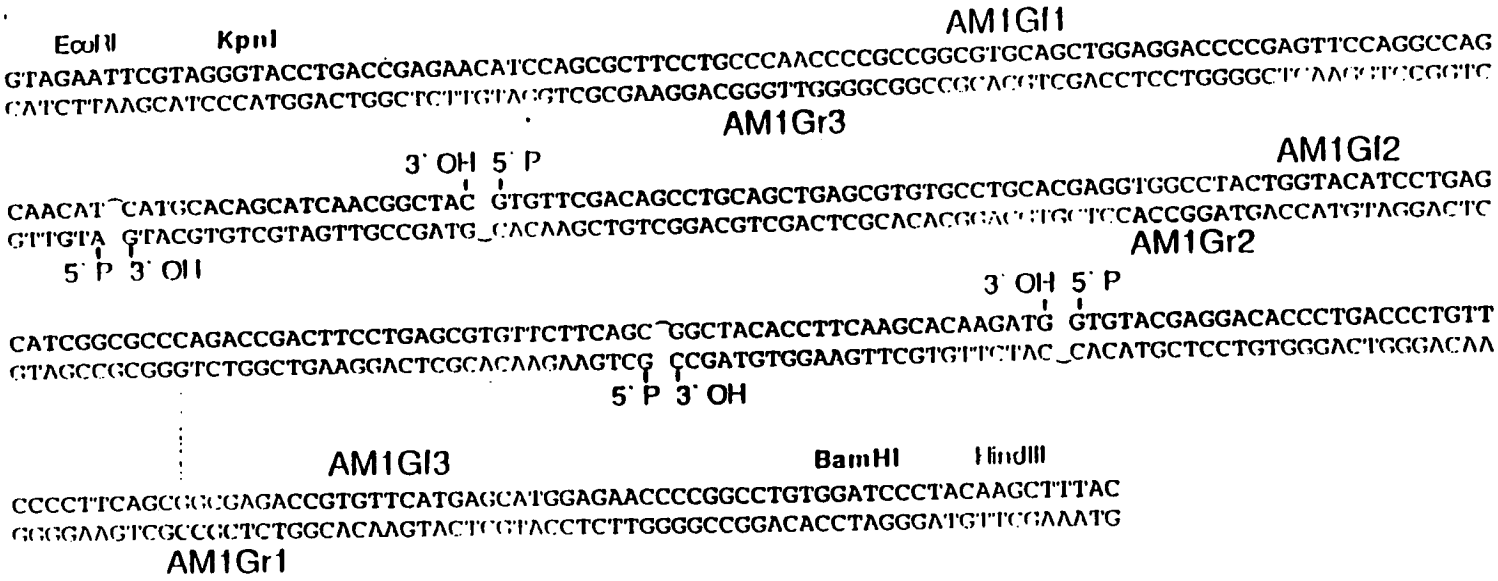


FIG. 5 (7 of 14)

Fragment H

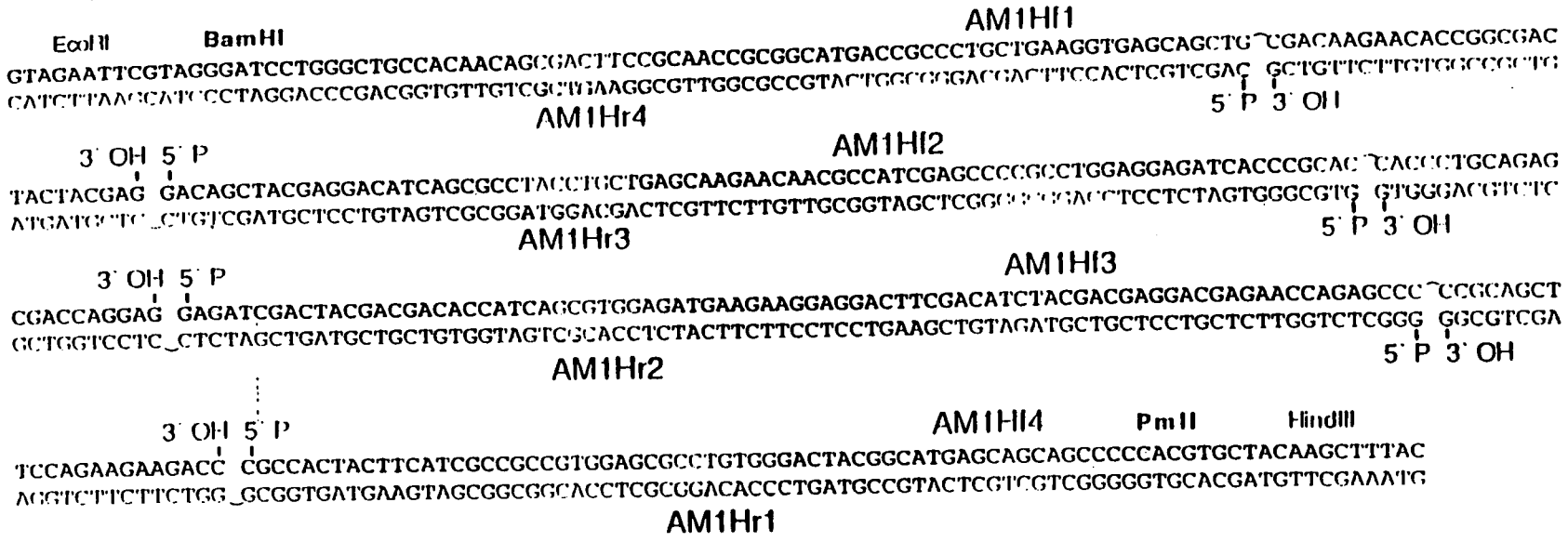


FIG. 5 (8 of 14)

Fragment I

FIG. 5 (9 of 14)

Fragment J

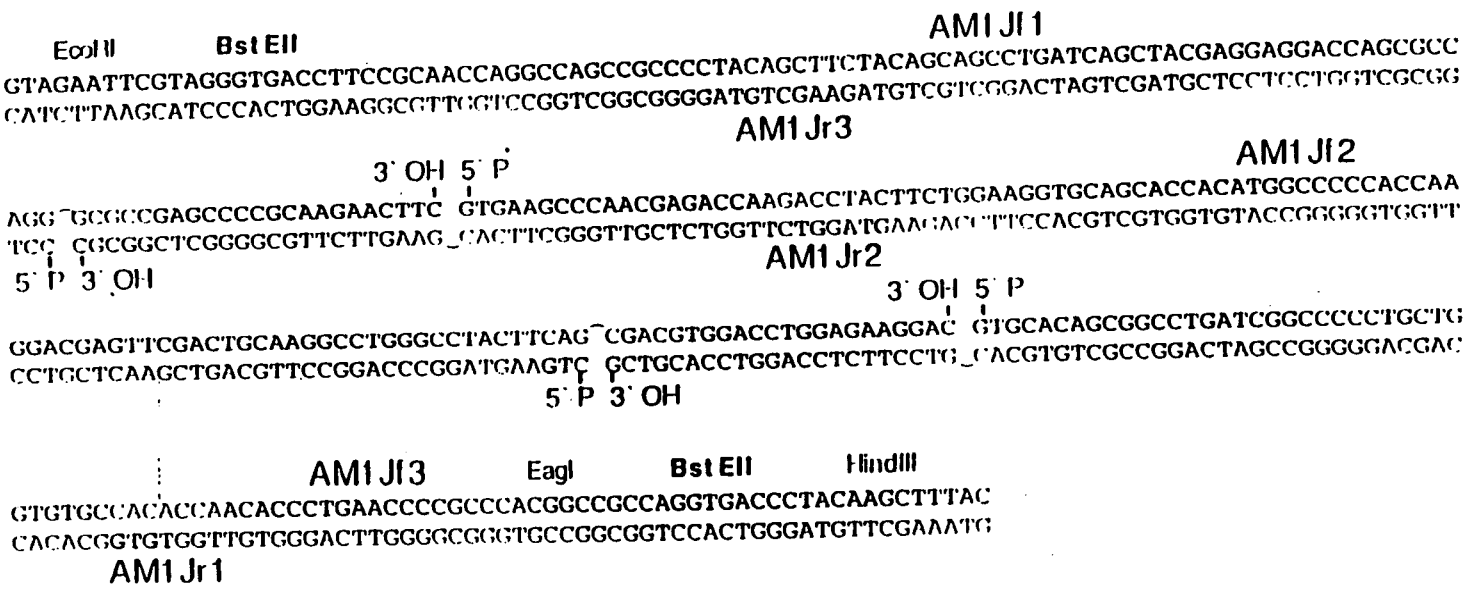


FIG. 5 (10 of 14)

Fragment K

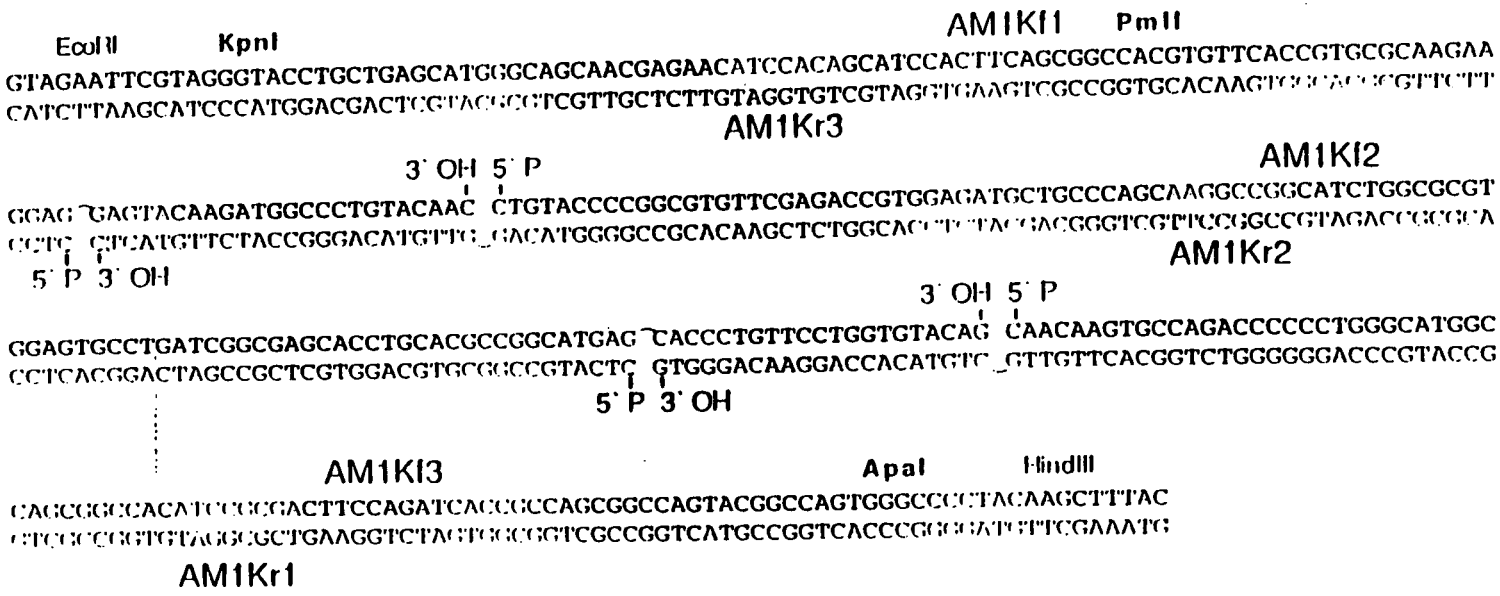


FIG. 5 (11 of 14)

Fragment L

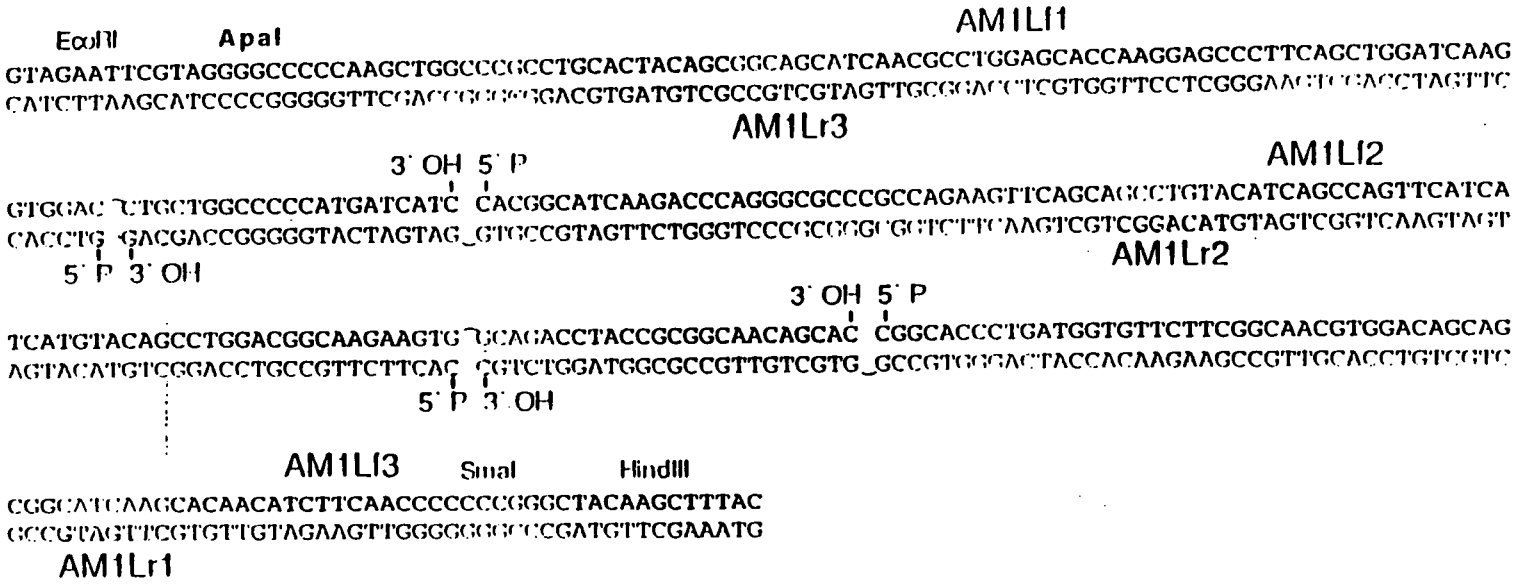


FIG. 5 (12 of 14)

Fragment M

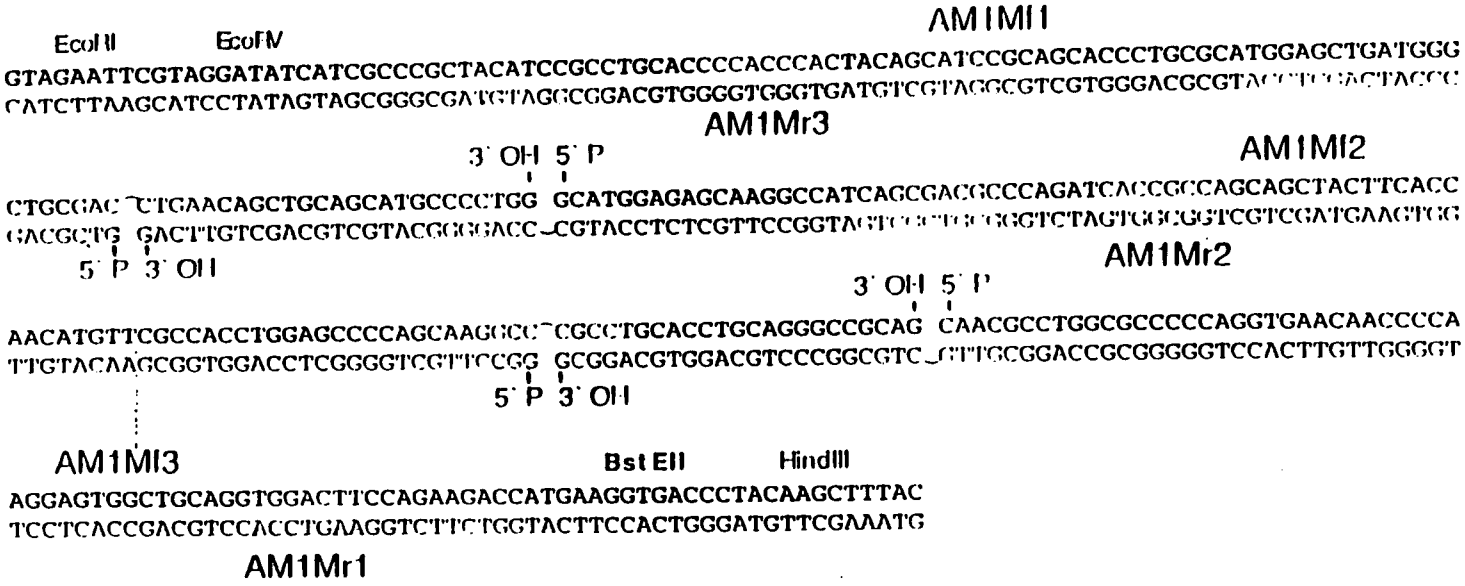


FIG. 5 (13 of 14)

Fragment N

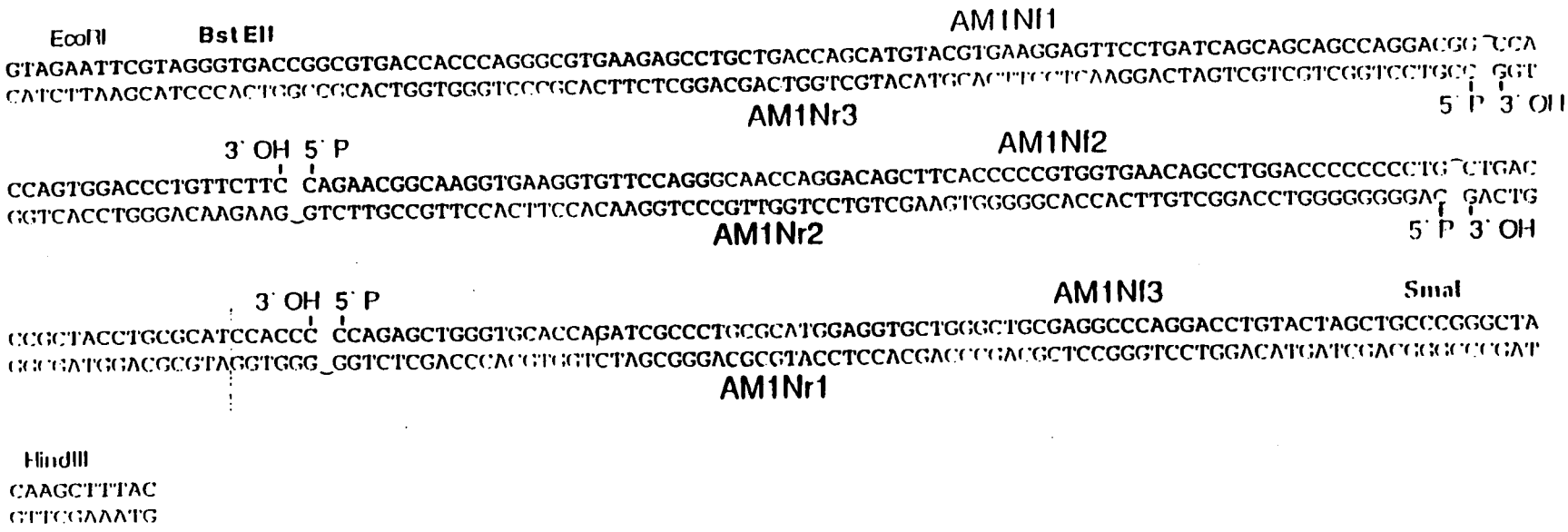


FIG. 5 (14 of 14)

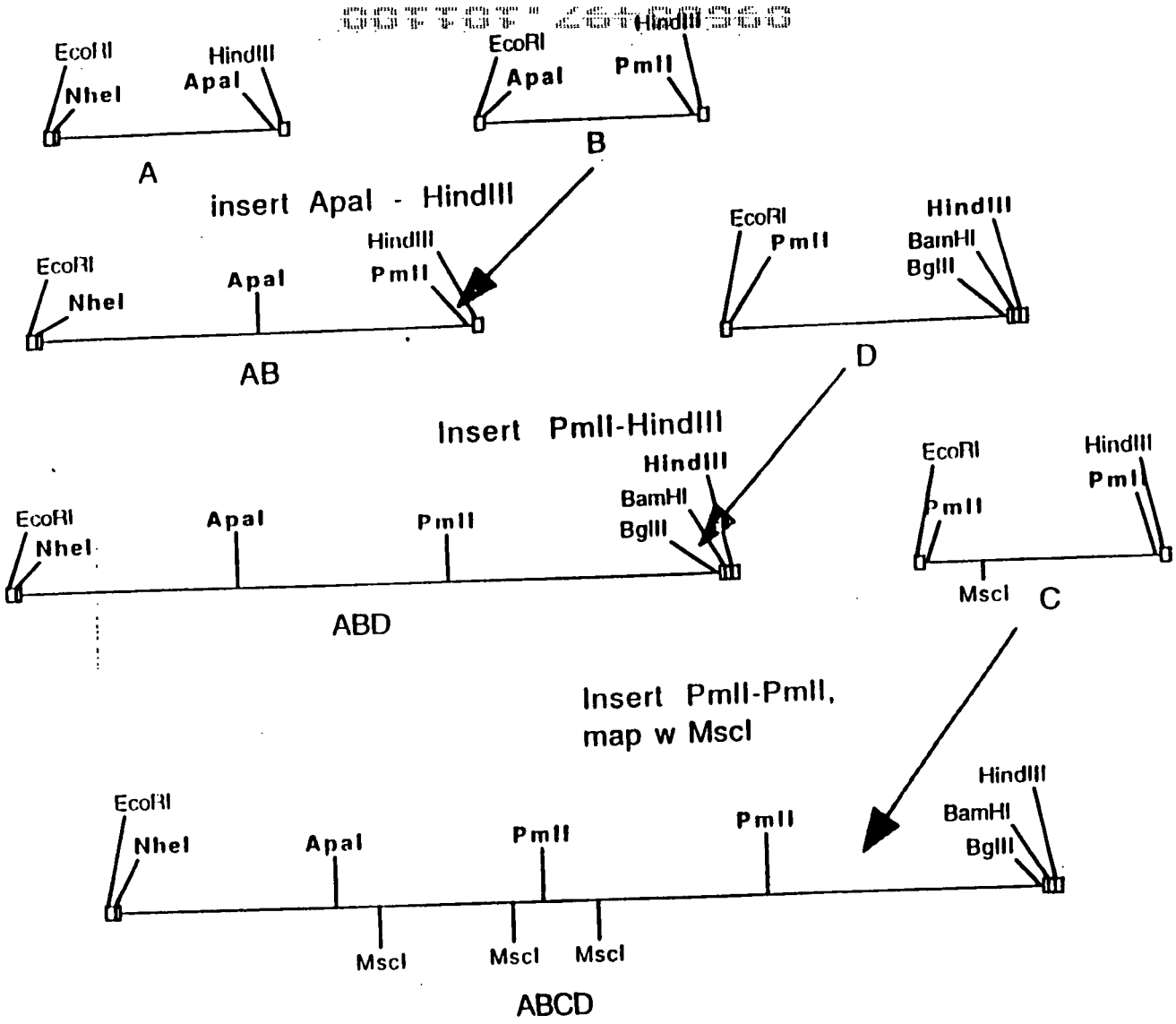

EcoRI
NheI
HindIII
ApaI
A
EcoRI
ApaI
PmlI
HindIII
B
insert ApaI - HindIII
EcoRI
NheI
ApaI
HindIII
PmlI
AB
EcoRI
PmlI
HindIII
BamHI
BglII
D
Insert PmlI-HindIII
EcoRI
NheI
ApaI
PmlI
HindIII
BamHI
BglII
ABD
EcoRI
PmlI
HindIII
PmlI
MscI
C
Insert PmlI-PmlI,
map w MscI
EcoRI
NheI
ApaI
PmlI
PmlI
HindIII
BamHI
BglII
MscI
MscI
MscI
ABCD

FIG. 6 (1 of 5)

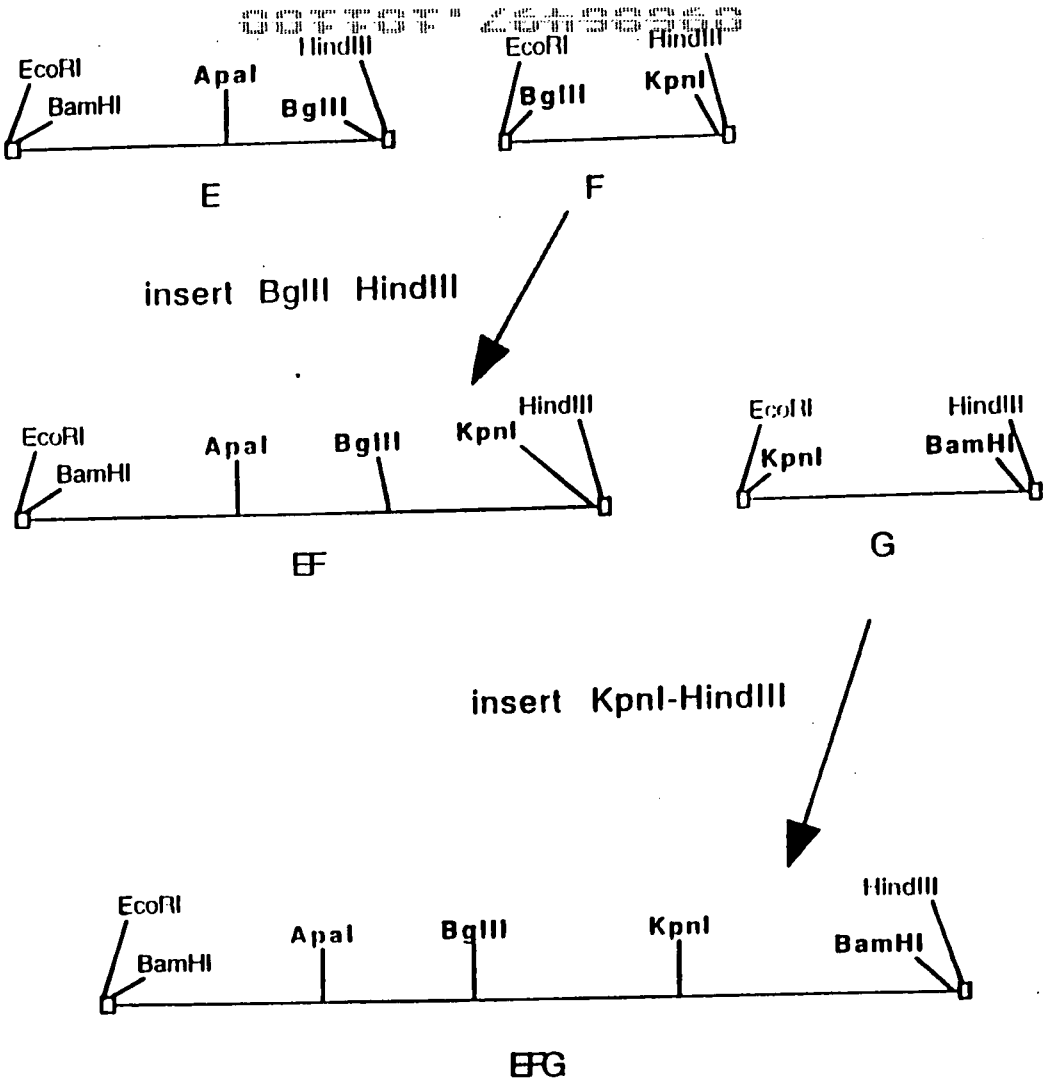

EcoRI
BamHI
ApaI
HindIII
BglII
E
EcoRI
BglII
HindIII
KpnI
F
insert BglII HindIII
EcoRI
BamHI
ApaI
BglII
KpnI
HindIII
EF
EcoRI
KpnI
HindIII
BamHI
G
insert KpnI-HindIII
EcoRI
BamHI
ApaI
BglII
KpnI
HindIII
BamHI
EFG

FIG. 6 (2 of 5)

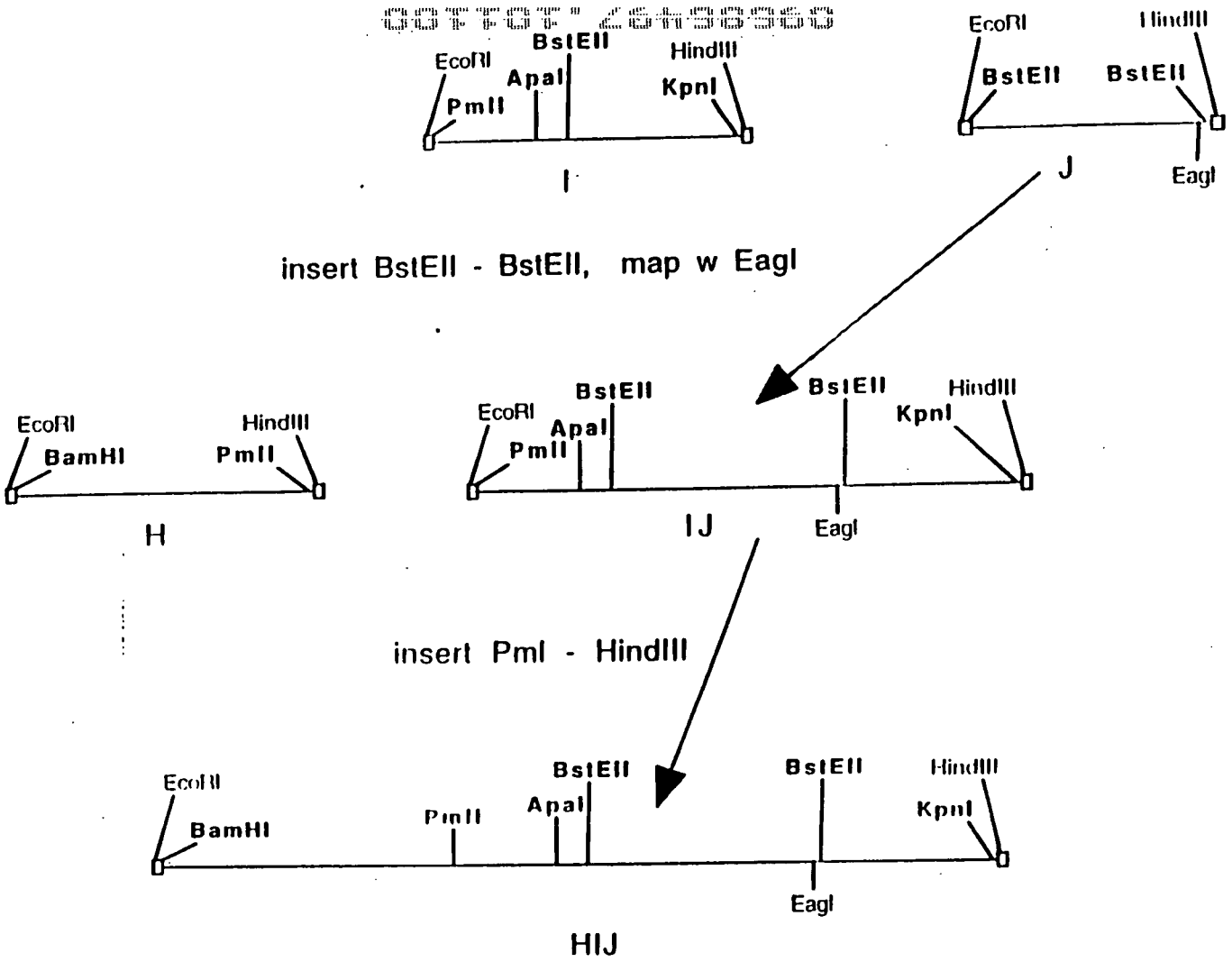

EcoRI
BstEII
HindIII
ApaI
PmlI
KpnI
I
EcoRI
HindIII
BstEII
BstEII
J
EagI
insert BstEII - BstEII, map w EagI
EcoRI
BamHI
HindIII
PmlI
H
EcoRI
BstEII
BstEII
HindIII
ApaI
PmlI
KpnI
IJ
EagI
insert PmI - HindIII
EcoRI
BstEII
BstEII
HindIII
BamHI
PmlI
ApaI
KpnI
EagI
HIJ

FIG. 6 (3 of 5)

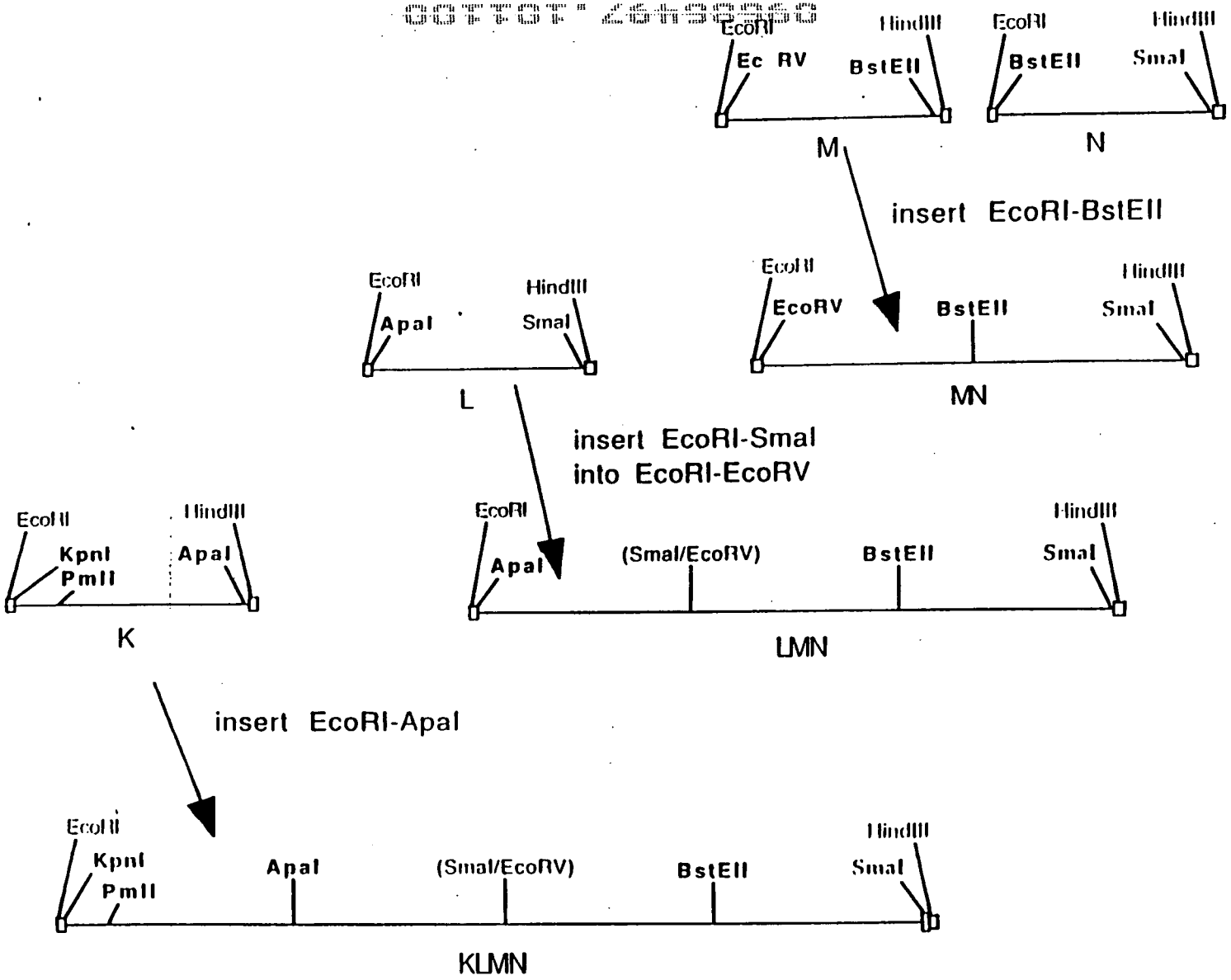

EcoRI
Ec RV
BstEII
HindIII
M
EcoRI
BstEII
SmaI
HindIII
N
insert EcoRI-BstEII
EcoRI
ApaI
SmaI
HindIII
L
EcoRI
EcoRV
BstEII
SmaI
HindIII
MN
insert EcoRI-SmaI
into EcoRI-EcoRV
EcoRI
KpnI
PmlI
ApaI
HindIII
K
EcoRI
ApaI
(SmaI/EcoRV)
BstEII
SmaI
HindIII
LMN
insert EcoRI-ApaI
EcoRI
KpnI
PmlI
ApaI
(SmaI/EcoRV)
BstEII
SmaI
HindIII
KLMN

FIG. 6 (4 of 5)

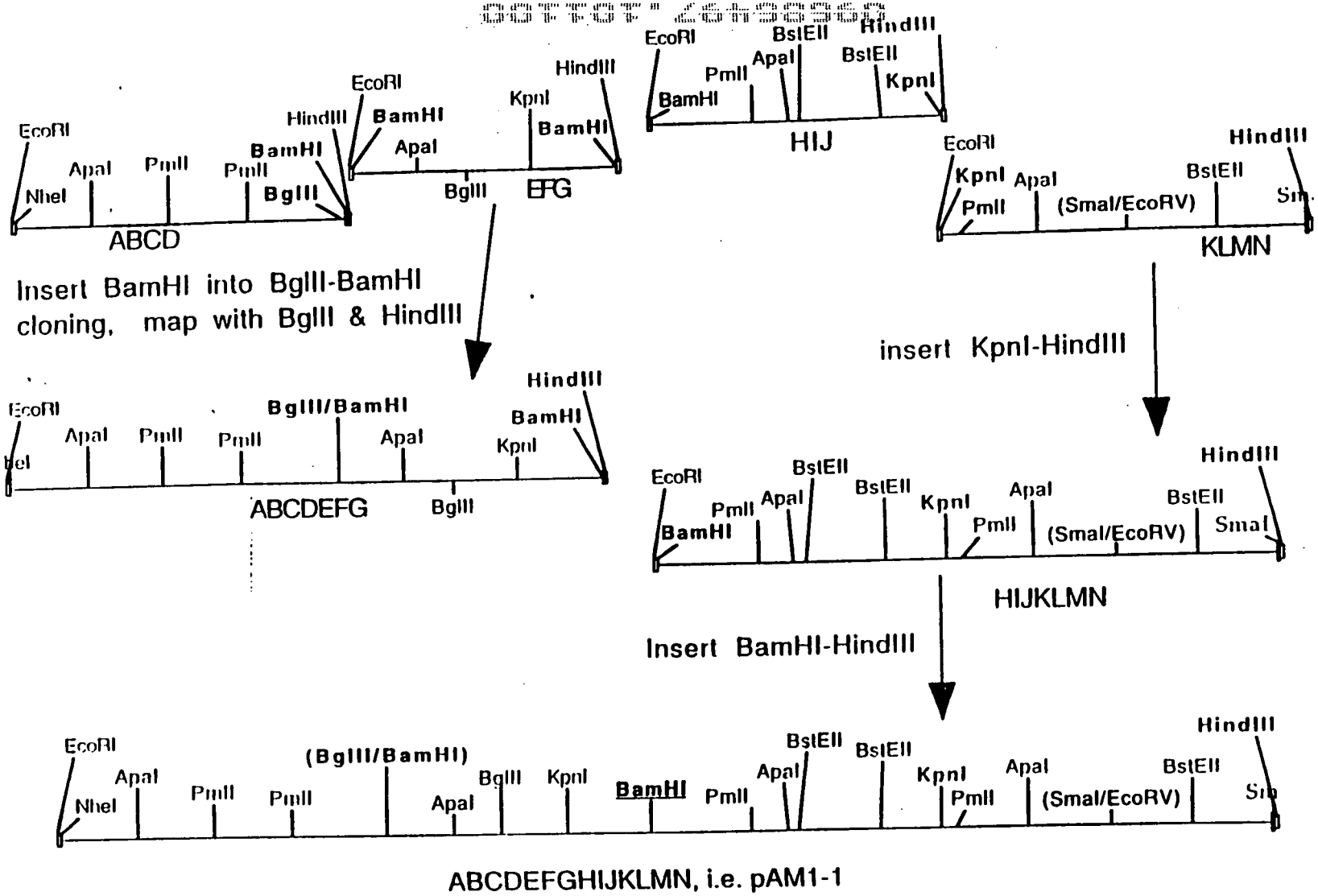

EcoRI
ApaI
PmlI
PmlI
NheI
BamHI
BglII
HindIII
EcoRI
BamHI
ApaI
KpnI
BamHI
HindIII
ABCD
BglII
EFG
EcoRI
BstEII
HindIII
PmlI
ApaI
BstEII
BamHI
KpnI
HIJ
EcoRI
KpnI
ApaI
HindIII
BstEII
PmlI
(SmaI/EcoRV)
KLMN
Insert BamHI into BglII-BamHI
cloning, map with BglII & HindIII
insert KpnI-HindIII
EcoRI
ApaI
PmlI
PmlI
BglII/BamHI
ApaI
HindIII
BamHI
KpnI
ABCDEFG
BglII
EcoRI
BstEII
HindIII
BamHI
PmlI
ApaI
BstEII
KpnI
ApaI
BstEII
PmlI
(SmaI/EcoRV)
SmaI
HIJKLMN
Insert BamHI-HindIII
EcoRI
ApaI
NheI
PmlI
PmlI
(BglII/BamHI)
ApaI
BglII
KpnI
BamHI
BstEII
PmlI
ApaI
BstEII
KpnI
PmlI
ApaI
(SmaI/EcoRV)
BstEII
HindIII
ABCDEFGHIJKLMN, i.e. pAM1-1

FIG. 6 (5 of 5)

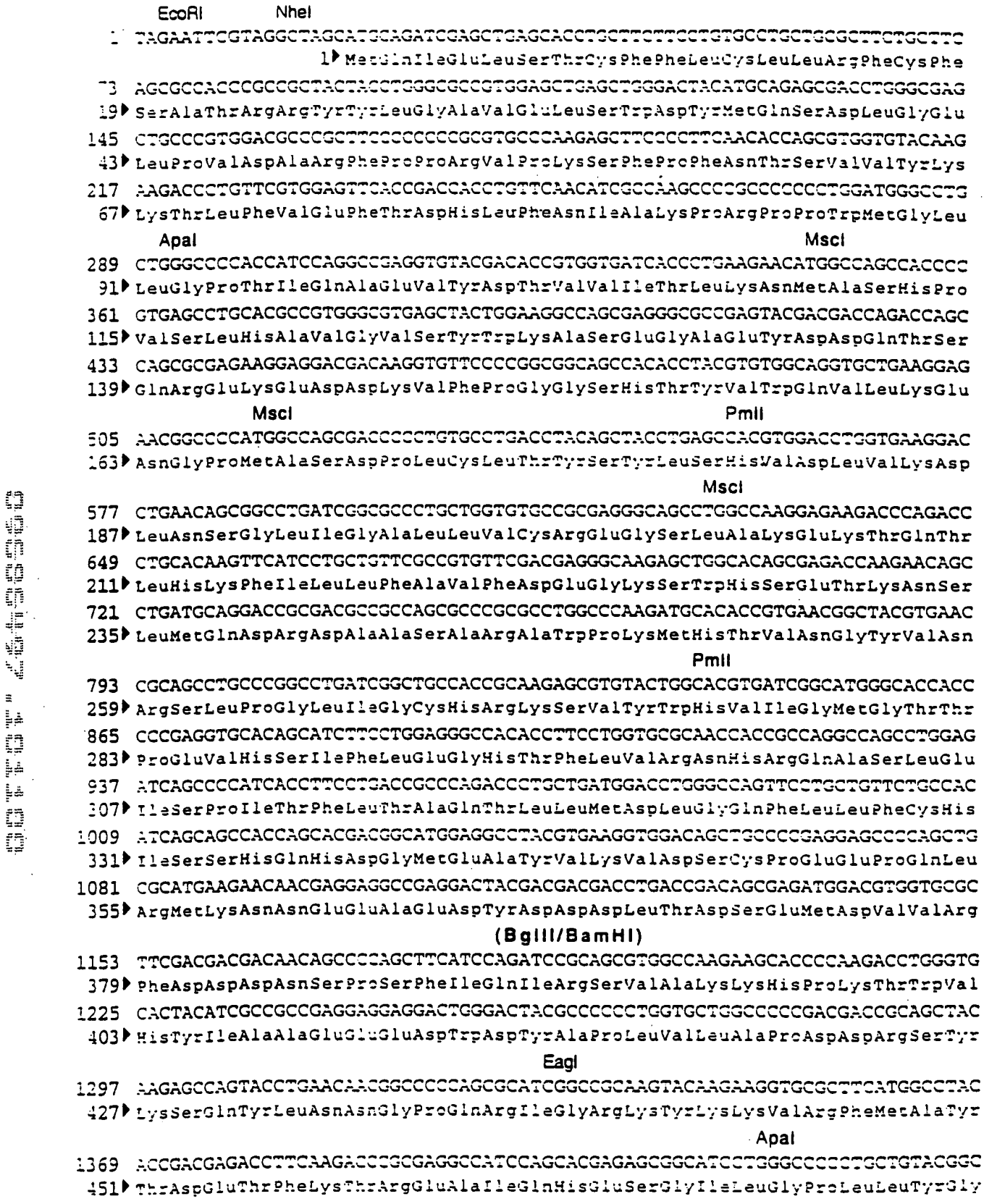

EcoRI
NheI
ApaI
MscI
MscI
PmlI
MscI
PmlI
(BglII/BamHI)
EagI
ApaI

FIG. 7 (1 of 3)

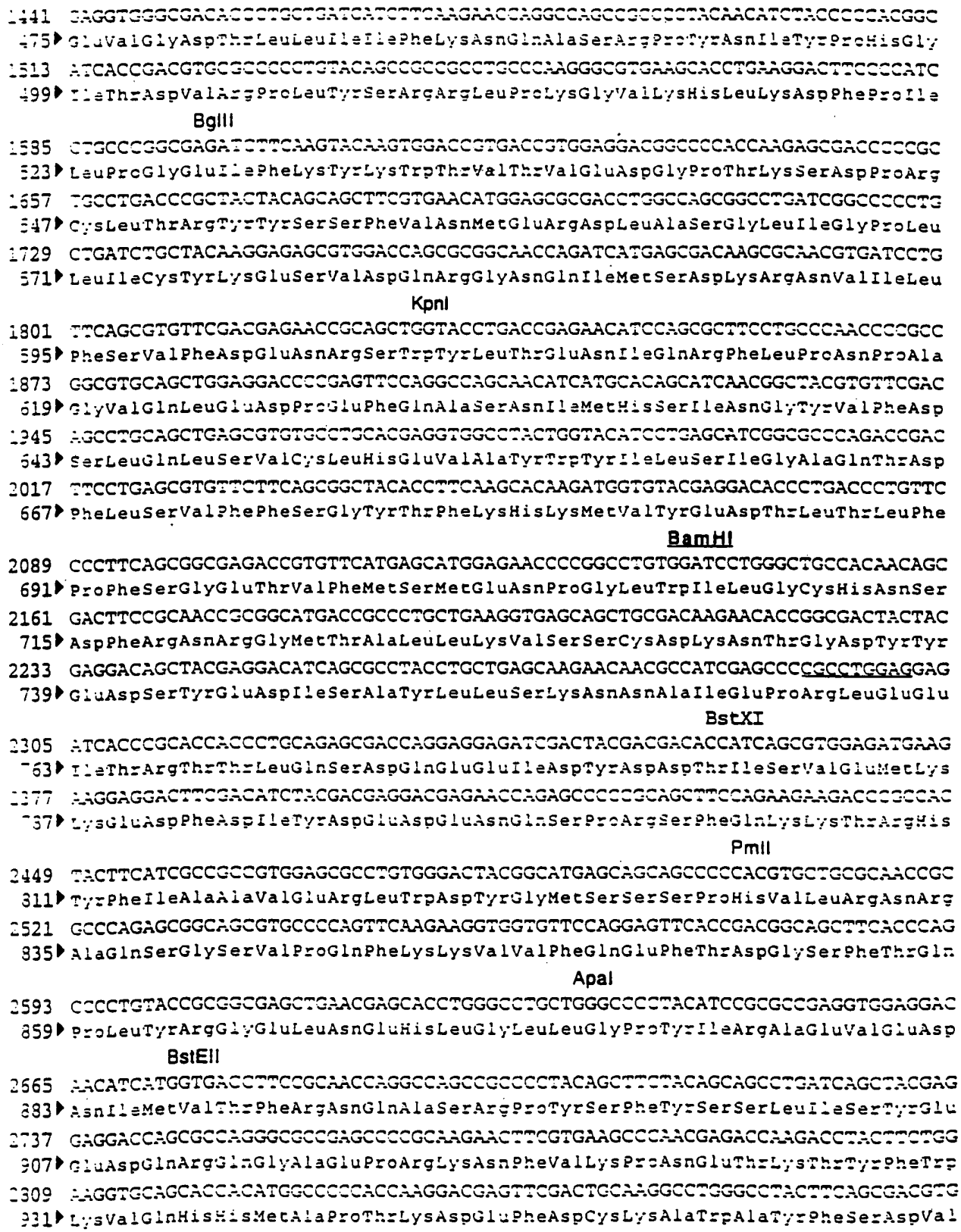

1441 GAGGTGGGCGACACCCTGCTGATCATCTTCAAGAACCAGGCCAGCCGCCCCTACAACATCTACCCCCACGGC
475 GluValGlyAspThrLeuLeuIleIlePheLysAsnGlnAlaSerArgProTyrAsnIleTyrProHisGly
1513 ATCACCGACGTGCGCCCCCTGTACAGCCGCCGCCTGCCCAAGGGCGTGAAGCACCTGAAGGACTTCCCCATC
499 IleThrAspValArgProLeuTyrSerArgArgLeuProLysGlyValLysHisLeuLysAspPheProIle
BglII
1585 CTGCCCGGCGAGATCTTCAAGTACAAGTGGACCGTGACCGTGGAGGACGGCCCCACCAAGAGCGACCCCCGC
523 LeuProGlyGluIlePheLysTyrLysTrpThrValThrValGluAspGlyProThrLysSerAspProArg
1657 TGCCTGACCCGCTACTACAGCAGCTTCGTGAACATGGAGCGCGACCTGGCCAGCGGCCTGATCGGCCCCCTG
547 CysLeuThrArgTyrTyrSerSerPheValAsnMetGluArgAspLeuAlaSerGlyLeuIleGlyProLeu
1729 CTGATCTGCTACAAGGAGAGCGTGGACCAGCGCGGCAACCAGATCATGAGCGACAAGCGCAACGTGATCCTG
571 LeuIleCysTyrLysGluSerValAspGlnArgGlyAsnGlnIleMetSerAspLysArgAsnValIleLeu
KpnI
1801 TTCAGCGTGTTCGACGAGAACCGCAGCTGGTACCTGACCGAGAACATCCAGCGCTTCCTGCCCAACCCCGCC
595 PheSerValPheAspGluAsnArgSerTrpTyrLeuThrGluAsnIleGlnArgPheLeuProAsnProAla
1873 GGCGTGCAGCTGGAGGACCCCGAGTTCCAGGCCAGCAACATCATGCACAGCATCAACGGCTACGTGTTCGAC
619 GlyValGlnLeuGluAspProGluPheGlnAlaSerAsnIleMetHisSerIleAsnGlyTyrValPheAsp
1945 AGCCTGCAGCTGAGCGTGTGCCTGCACGAGGTGGCCTACTGGTACATCCTGAGCATCGGCGCCCAGACCGAC
643 SerLeuGlnLeuSerValCysLeuHisGluValAlaTyrTrpTyrIleLeuSerIleGlyAlaGlnThrAsp
2017 TTCCTGAGCGTGTTCTTCAGCGGCTACACCTTCAAGCACAAGATGGTGTACGAGGACACCCTGACCCTGTTC
667 PheLeuSerValPhePheSerGlyTyrThrPheLysHisLysMetValTyrGluAspThrLeuThrLeuPhe
BamHI
2089 CCCTTCAGCGGCGAGACCGTGTTCATGAGCATGGAGAACCCCGGCCTGTGGATCCTGGGCTGCCACAACAGC
691 ProPheSerGlyGluThrValPheMetSerMetGluAsnProGlyLeuTrpIleLeuGlyCysHisAsnSer
2161 GACTTCCGCAACCGCGGCATGACCGCCCTGCTGAAGGTGAGCAGCTGCGACAAGAACACCGGCGACTACTAC
715 AspPheArgAsnArgGlyMetThrAlaLeuLeuLysValSerSerCysAspLysAsnThrGlyAspTyrTyr
2233 GAGGACAGCTACGAGGACATCAGCGCCTACCTGCTGAGCAAGAACAACGCCATCGAGCCCCGCCTGGAGGAG
739 GluAspSerTyrGluAspIleSerAlaTyrLeuLeuSerLysAsnAsnAlaIleGluProArgLeuGluGlu
BstXI
2305 ATCACCCGCACCACCCTGCAGAGCGACCAGGAGGAGATCGACTACGACGACACCATCAGCGTGGAGATGAAG
763 IleThrArgThrThrLeuGlnSerAspGlnGluGluIleAspTyrAspAspThrIleSerValGluMetLys
2377 AAGGAGGACTTCGACATCTACGACGAGGACGAGAACCAGAGCCCCCGCAGCTTCCAGAAGAAGACCCGCCAC
787 LysGluAspPheAspIleTyrAspGluAspGluAsnGlnSerProArgSerPheGlnLysLysThrArgHis
PmlI
2449 TACTTCATCGCCGCCGTGGAGCGCCTGTGGGACTACGGCATGAGCAGCAGCCCCCACGTGCTGCGCAACCGC
811 TyrPheIleAlaAlaValGluArgLeuTrpAspTyrGlyMetSerSerSerProHisValLeuArgAsnArg
2521 GCCCAGAGCGGCAGCGTGCCCCAGTTCAAGAAGGTGGTGTTCCAGGAGTTCACCGACGGCAGCTTCACCCAG
835 AlaGlnSerGlySerValProGlnPheLysLysValValPheGlnGluPheThrAspGlySerPheThrGln
ApaI
2593 CCCCTGTACCGCGGCGAGCTGAACGAGCACCTGGGCCTGCTGGGCCCCTACATCCGCGCCGAGGTGGAGGAC
859 ProLeuTyrArgGlyGluLeuAsnGluHisLeuGlyLeuLeuGlyProTyrIleArgAlaGluValGluAsp
BstEII
2665 AACATCATGGTGACCTTCCGCAACCAGGCCAGCCGCCCCTACAGCTTCTACAGCAGCCTGATCAGCTACGAG
883 AsnIleMetValThrPheArgAsnGlnAlaSerArgProTyrSerPheTyrSerSerLeuIleSerTyrGlu
2737 GAGGACCAGCGCCAGGGCGCCGAGCCCCGCAAGAACTTCGTGAAGCCCAACGAGACCAAGACCTACTTCTGG
907 GluAspGlnArgGlnGlyAlaGluProArgLysAsnPheValLysProAsnGluThrLysThrTyrPheTrp
2809 AAGGTGCAGCACCACATGGCCCCCACCAAGGACGAGTTCGACTGCAAGGCCTGGGCCTACTTCAGCGACGTG
931 LysValGlnHisHisMetAlaProThrLysAspGluPheAspCysLysAlaTrpAlaTyrPheSerAspVal

FIG. 7 (2 of 3)

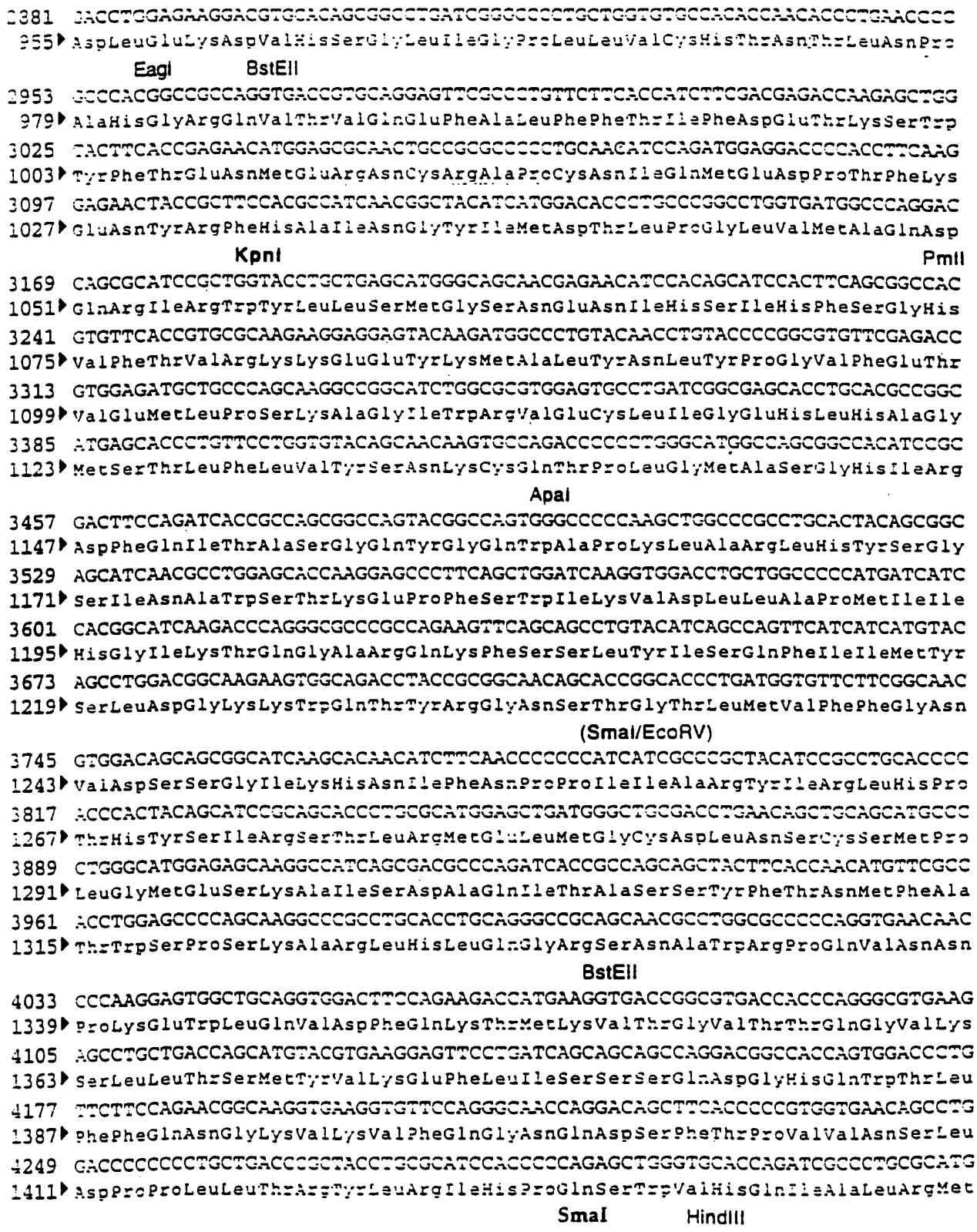
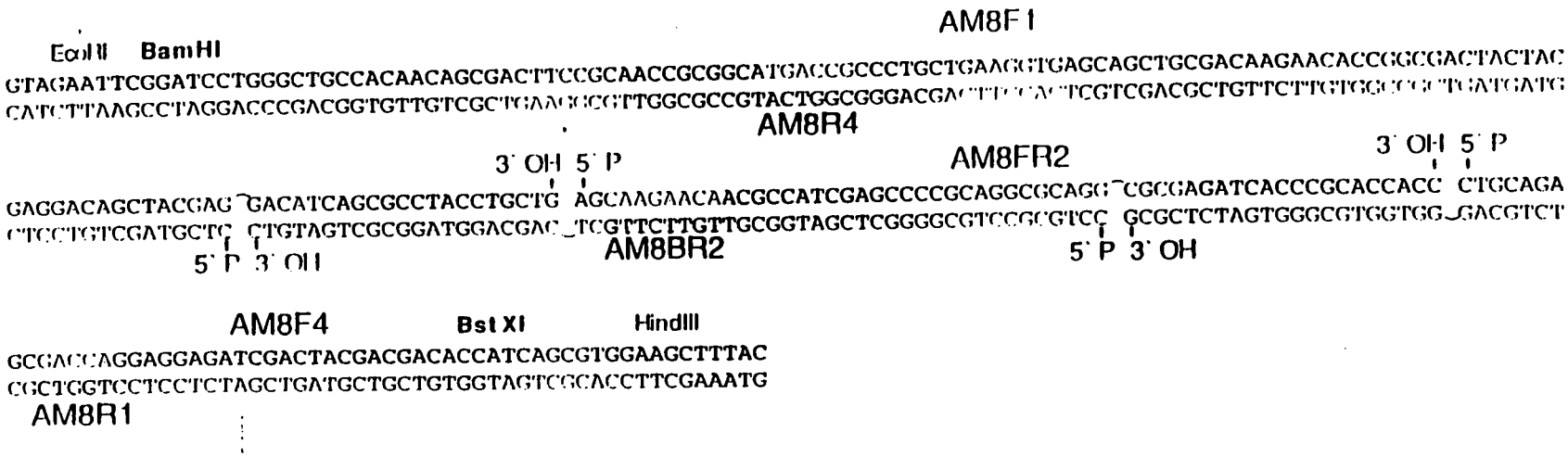

2381 GACCTGGAGAAGGACGTGCACAGCGGCCTGATCGGGCCCCTGCTGGTGTGCCACACCAACACCCTGAACCCC
955 AspLeuGluLysAspValHisSerGlyLeuIleGlyProLeuLeuValCysHisThrAsnThrLeuAsnPro
EagI
BstEII
2953 GCCCACGGCCGCCAGGTGACCGTGCAGGAGTTCGCCCTGTTCTTCACCATCTTCGACGAGACCAAGAGCTGG
979 AlaHisGlyArgGlnValThrValGlnGluPheAlaLeuPhePheThrIlePheAspGluThrLysSerTrp
3025 TACTTCACCGAGAACATGGAGCGCAACTGCCGCGCCCCCTGCAACATCCAGATGGAGGACCCCACCTTCAAG
1003 TyrPheThrGluAsnMetGluArgAsnCysArgAlaProCysAsnIleGlnMetGluAspProThrPheLys
3097 GAGAACTACCGCTTCCACGCCATCAACGGCTACATCATGGACACCCTGCCCGGCCTGGTGATGGCCCAGGAC
1027 GluAsnTyrArgPheHisAlaIleAsnGlyTyrIleMetAspThrLeuProGlyLeuValMetAlaGlnAsp
KpnI
PmlI
3169 CAGCGCATCCGCTGGTACCTGCTGAGCATGGGCAGCAACGAGAACATCCACAGCATCCACTTCAGCGGCCAC
1051 GlnArgIleArgTrpTyrLeuLeuSerMetGlySerAsnGluAsnIleHisSerIleHisPheSerGlyHis
3241 GTGTTCACCGTGCGCAAGAAGGAGGAGTACAAGATGGCCCTGTACAACCTGTACCCCGGCGTGTTCGAGACC
1075 ValPheThrValArgLysLysGluGluTyrLysMetAlaLeuTyrAsnLeuTyrProGlyValPheGluThr
3313 GTGGAGATGCTGCCCAGCAAGGCCGGCATCTGGCGCGTGGAGTGCCTGATCGGCGAGCACCTGCACGCCGGC
1099 ValGluMetLeuProSerLysAlaGlyIleTrpArgValGluCysLeuIleGlyGluHisLeuHisAlaGly
3385 ATGAGCACCCTGTTCCTGGTGTACAGCAACAAGTGCCAGACCCCCCTGGGCATGGCCAGCGGCCACATCCGC
1123 MetSerThrLeuPheLeuValTyrSerAsnLysCysGlnThrProLeuGlyMetAlaSerGlyHisIleArg
ApaI
3457 GACTTCCAGATCACCGCCAGCGGCCAGTACGGCCAGTGGGCCCCCAAGCTGGCCCGCCTGCACTACAGCGGC
1147 AspPheGlnIleThrAlaSerGlyGlnTyrGlyGlnTrpAlaProLysLeuAlaArgLeuHisTyrSerGly
3529 AGCATCAACGCCTGGAGCACCAAGGAGCCCTTCAGCTGGATCAAGGTGGACCTGCTGGCCCCCATGATCATC
1171 SerIleAsnAlaTrpSerThrLysGluProPheSerTrpIleLysValAspLeuLeuAlaProMetIleIle
3601 CACGGCATCAAGACCCAGGGCGCCCGCCAGAAGTTCAGCAGCCTGTACATCAGCCAGTTCATCATCATGTAC
1195 HisGlyIleLysThrGlnGlyAlaArgGlnLysPheSerSerLeuTyrIleSerGlnPheIleIleMetTyr
3673 AGCCTGGACGGCAAGAAGTGGCAGACCTACCGCGGCAACAGCACCGGCACCCTGATGGTGTTCTTCGGCAAC
1219 SerLeuAspGlyLysLysTrpGlnThrTyrArgGlyAsnSerThrGlyThrLeuMetValPhePheGlyAsn
(SmaI/EcoRV)
3745 GTGGACAGCAGCGGCATCAAGCACAACATCTTCAACCCCCCCATCATCGCCCGCTACATCCGCCTGCACCCC
1243 ValAspSerSerGlyIleLysHisAsnIlePheAsnProProIleIleAlaArgTyrIleArgLeuHisPro
3817 ACCCACTACAGCATCCGCAGCACCCTGCGCATGGAGCTGATGGGCTGCGACCTGAACAGCTGCAGCATGCCC
1267 ThrHisTyrSerIleArgSerThrLeuArgMetGluLeuMetGlyCysAspLeuAsnSerCysSerMetPro
3889 CTGGGCATGGAGAGCAAGGCCATCAGCGACGCCCAGATCACCGCCAGCAGCTACTTCACCAACATGTTCGCC
1291 LeuGlyMetGluSerLysAlaIleSerAspAlaGlnIleThrAlaSerSerTyrPheThrAsnMetPheAla
3961 ACCTGGAGCCCCAGCAAGGCCCGCCTGCACCTGCAGGGCCGCAGCAACGCCTGGCGCCCCCAGGTGAACAAC
1315 ThrTrpSerProSerLysAlaArgLeuHisLeuGlnGlyArgSerAsnAlaTrpArgProGlnValAsnAsn
BstEII
4033 CCCAAGGAGTGGCTGCAGGTGGACTTCCAGAAGACCATGAAGGTGACCGGCGTGACCACCCAGGGCGTGAAG
1339 ProLysGluTrpLeuGlnValAspPheGlnLysThrMetLysValThrGlyValThrThrGlnGlyValLys
4105 AGCCTGCTGACCAGCATGTACGTGAAGGAGTTCCTGATCAGCAGCAGCCAGGACGGCCACCAGTGGACCCTG
1363 SerLeuLeuThrSerMetTyrValLysGluPheLeuIleSerSerSerGlnAspGlyHisGlnTrpThrLeu
4177 TTCTTCCAGAACGGCAAGGTGAAGGTGTTCCAGGGCAACCAGGACAGCTTCACCCCCGTGGTGAACAGCCTG
1387 PhePheGlnAsnGlyLysValLysValPheGlnGlyAsnGlnAspSerPheThrProValValAsnSerLeu
4249 GACCCCCCCCTGCTGACCCGCTACCTGCGCATCCACCCCCAGAGCTGGGTGCACCAGATCGCCCTGCGCATG
1411 AspProProLeuLeuThrArgTyrLeuArgIleHisProGlnSerTrpValHisGlnIleAlaLeuArgMet
SmaI
HindIII
4321 GAGGTGCTGGGCTGCGAGGCCCAGGACCTGTACTAGCTGCCCGGGCTACAAGCTTT
1435 GluValLeuGlyCysGluAlaGlnAspLeuTyr•••

FIG. 7 (3 of 3)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EcoRI
BamHI
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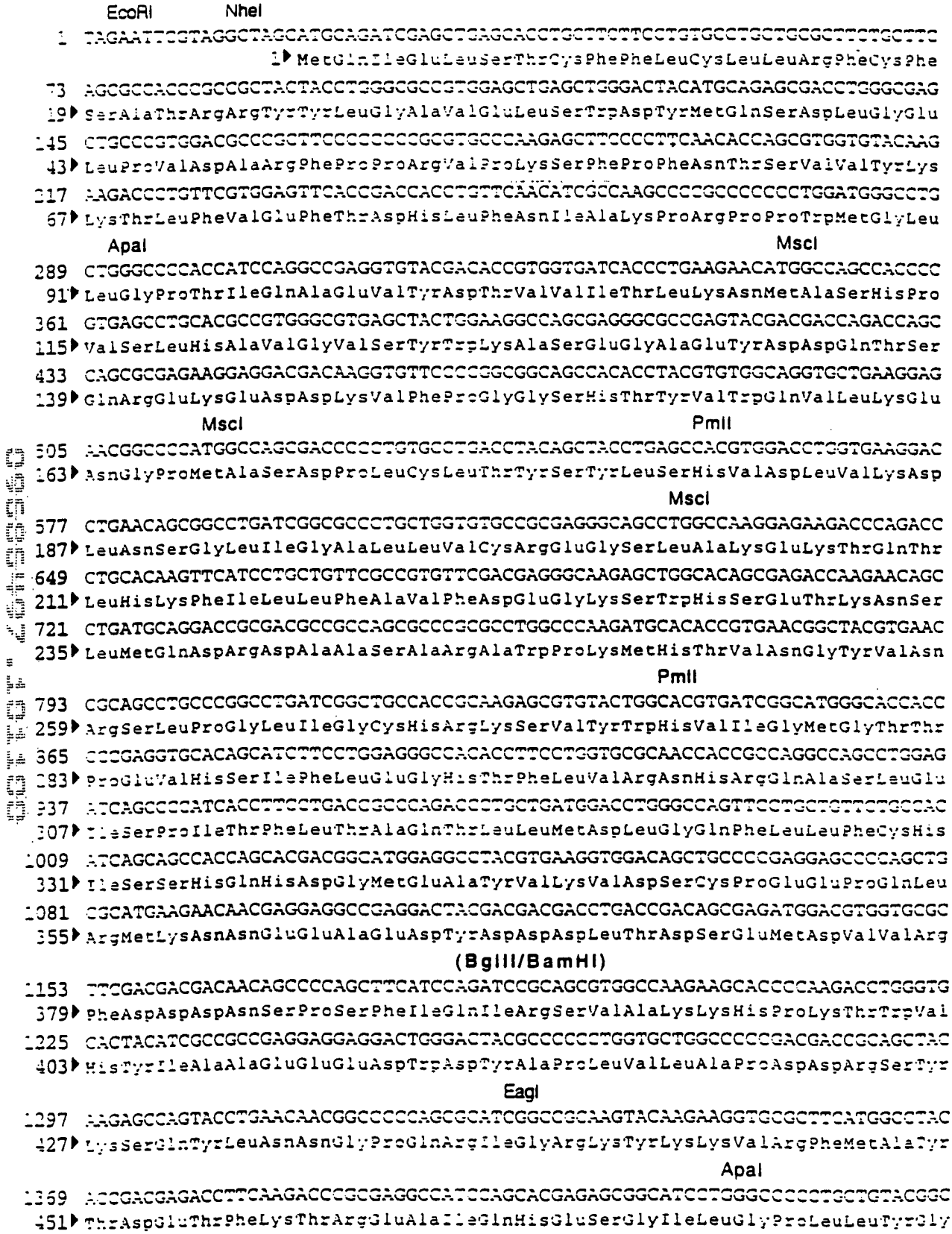
CATCTTAAGCCTAGGACCCGACGGTGTTGTCGCTGAAGGCGTTGGCGCCGTACTGGCGGGACGACTTCCACTCGTCGACGCTGTTCTTGTGGCCGCTGATGATG
AM8R4
3' OH 5' P
AM8FR2
3' OH 5' P
GAGGACAGCTACGAGGACATCAGCGCCTACCTGCTGAGCAAGAACAACGCCATCGAGCCCCGCAGGCGCAGCTCGCGAGATCACCCGCACCACC CTGCAGA
CTCCTGTCGATGCTCCTGTAGTCGCGGATGGACGACTCGTTCTTGTTGCGGTAGCTCGGGGCGTCCGCGTCC GCGCTCTAGTGGGCGTGGTGG GACGTCT
5' P 3' OH
AM8BR2
5' P 3' OH
AM8F4
BstXI
HindIII
GCCACCAGGAGGAGATCGACTACGACGACACCATCAGCGTGGAAGCTTTAC
CGGTGGTCCTCCTCTAGCTGATGCTGCTGTGGTAGTCGCACCTTCGAAATG
AM8R1

FIG. 8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NheI
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LeuProValAspAlaArgPheProProArgValProLysSerPheProPheAsnThrSerValValTyrLys
LysThrLeuPheValGluPheThrAspHisLeuPheAsnIleAlaLysProArgProProTrpMetGlyLeu
ApaI
MscI
LeuGlyProThrIleGlnAlaGluValTyrAspThrValValIleThrLeuLysAsnMetAlaSerHisPro
ValSerLeuHisAlaValGlyValSerTyrTrpLysAlaSerGluGlyAlaGluTyrAspAspGlnThrSer
GlnArgGluLysGluAspAspLysValPheProGlyGlySerHisThrTyrValTrpGlnValLeuLysGlu
MscI
PmlI
AsnGlyProMetAlaSerAspProLeuCysLeuThrTyrSerTyrLeuSerHisValAspLeuValLysAsp
MscI
LeuAsnSerGlyLeuIleGlyAlaLeuLeuValCysArgGluGlySerLeuAlaLysGluLysThrGlnThr
LeuHisLysPheIleLeuLeuPheAlaValPheAspGluGlyLysSerTrpHisSerGluThrLysAsnSer
LeuMetGlnAspArgAspAlaAlaSerAlaArgAlaTrpProLysMetHisThrValAsnGlyTyrValAsn
PmlI
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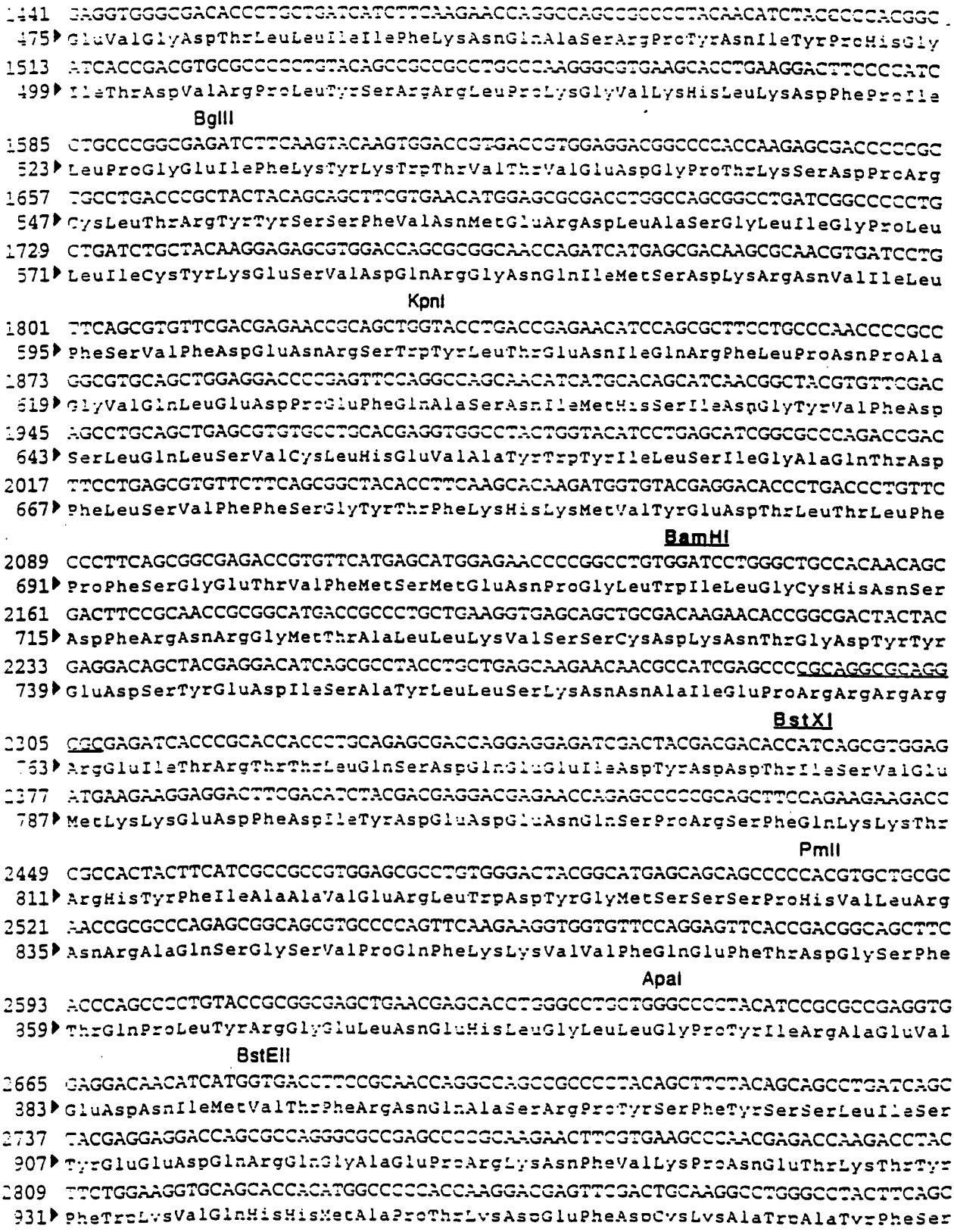
ProGluValHisSerIlePheLeuGluGlyHisThrPheLeuValArgAsnHisArgGlnAlaSerLeuGlu
IleSerProIleThrPheLeuThrAlaGlnThrLeuLeuMetAspLeuGlyGlnPheLeuLeuPheCysHis
IleSerSerHisGlnHisAspGlyMetGluAlaTyrValLysValAspSerCysProGluGluProGlnLeu
ArgMetLysAsnAsnGluGluAlaGluAspTyrAspAspAspLeuThrAspSerGluMetAspValValArg
(BglII/BamHI)
PheAspAspAspAsnSerProSerPheIleGlnIleArgSerValAlaLysLysHisProLysThrTrpVal
HisTyrIleAlaAlaGluGluGluAspTrpAspTyrAlaProLeuValLeuAlaProAspAspArgSerTyr
EagI
LysSerGlnTyrLeuAsnAsnGlyProGlnArgIleGlyArgLysTyrLysLysValArgPheMetAlaTyr
ApaI
ThrAspGluThrPheLysThrArgGluAlaIleGlnHisGluSerGlyIleLeuGlyProLeuLeuTyrGly

FIG. 9 (1 of 3)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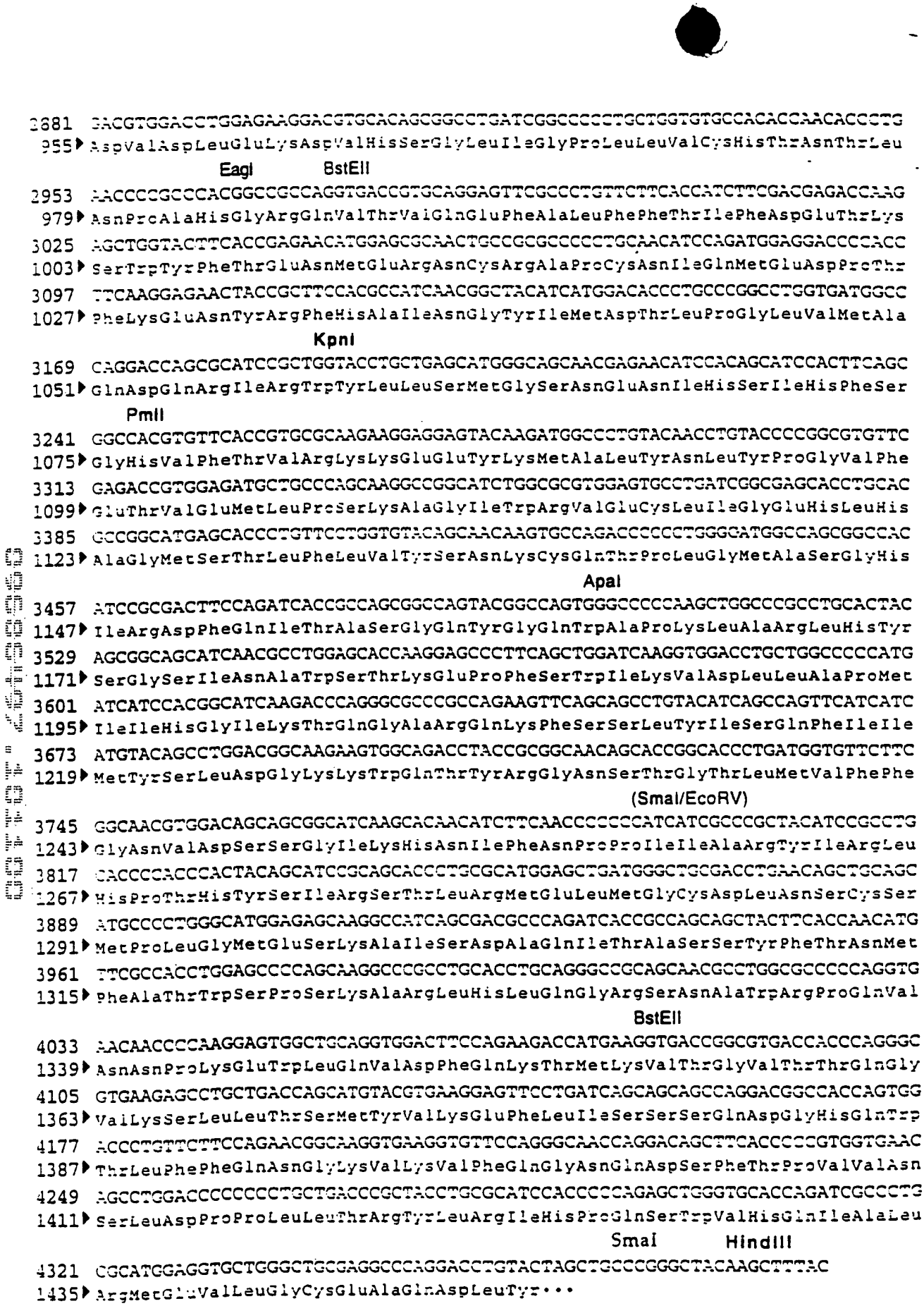
1513 ATCACCGACGTGCGCCCCCTGTACAGCCGCCGCCTGCCCAAGGGCGTGAAGCACCTGAAGGACTTCCCCATC
499 IleThrAspValArgProLeuTyrSerArgArgLeuProLysGlyValLysHisLeuLysAspPheProIle
BglII
1585 CTGCCCGGCGAGATCTTCAAGTACAAGTGGACCGTGACCGTGGAGGACGGCCCCACCAAGAGCGACCCCCGC
523 LeuProGlyGluIlePheLysTyrLysTrpThrValThrValGluAspGlyProThrLysSerAspProArg
1657 TGCCTGACCCGCTACTACAGCAGCTTCGTGAACATGGAGCGCGACCTGGCCAGCGGCCTGATCGGCCCCCTG
547 CysLeuThrArgTyrTyrSerSerPheValAsnMetGluArgAspLeuAlaSerGlyLeuIleGlyProLeu
1729 CTGATCTGCTACAAGGAGAGCGTGGACCAGCGCGGCAACCAGATCATGAGCGACAAGCGCAACGTGATCCTG
571 LeuIleCysTyrLysGluSerValAspGlnArgGlyAsnGlnIleMetSerAspLysArgAsnValIleLeu
KpnI
1801 TTCAGCGTGTTCGACGAGAACCGCAGCTGGTACCTGACCGAGAACATCCAGCGCTTCCTGCCCAACCCCGCC
595 PheSerValPheAspGluAsnArgSerTrpTyrLeuThrGluAsnIleGlnArgPheLeuProAsnProAla
1873 GGCGTGCAGCTGGAGGACCCCGAGTTCCAGGCCAGCAACATCATGCACAGCATCAACGGCTACGTGTTCGAC
619 GlyValGlnLeuGluAspProGluPheGlnAlaSerAsnIleMetHisSerIleAsnGlyTyrValPheAsp
1945 AGCCTGCAGCTGAGCGTGTGCCTGCACGAGGTGGCCTACTGGTACATCCTGAGCATCGGCGCCCAGACCGAC
643 SerLeuGlnLeuSerValCysLeuHisGluValAlaTyrTrpTyrIleLeuSerIleGlyAlaGlnThrAsp
2017 TTCCTGAGCGTGTTCTTCAGCGGCTACACCTTCAAGCACAAGATGGTGTACGAGGACACCCTGACCCTGTTC
667 PheLeuSerValPhePheSerGlyTyrThrPheLysHisLysMetValTyrGluAspThrLeuThrLeuPhe
BamHI
2089 CCCTTCAGCGGCGAGACCGTGTTCATGAGCATGGAGAACCCCGGCCTGTGGATCCTGGGCTGCCACAACAGC
691 ProPheSerGlyGluThrValPheMetSerMetGluAsnProGlyLeuTrpIleLeuGlyCysHisAsnSer
2161 GACTTCCGCAACCGCGGCATGACCGCCCTGCTGAAGGTGAGCAGCTGCGACAAGAACACCGGCGACTACTAC
715 AspPheArgAsnArgGlyMetThrAlaLeuLeuLysValSerSerCysAspLysAsnThrGlyAspTyrTyr
2233 GAGGACAGCTACGAGGACATCAGCGCCTACCTGCTGAGCAAGAACAACGCCATCGAGCCCCGCAGGCGCAGG
739 GluAspSerTyrGluAspIleSerAlaTyrLeuLeuSerLysAsnAsnAlaIleGluProArgArgArgArg
BstXI
2305 CGCGAGATCACCCGCACCACCCTGCAGAGCGACCAGGAGGAGATCGACTACGACGACACCATCAGCGTGGAG
763 ArgGluIleThrArgThrThrLeuGlnSerAspGlnGluGluIleAspTyrAspAspThrIleSerValGlu
2377 ATGAAGAAGGAGGACTTCGACATCTACGACGAGGACGAGAACCAGAGCCCCCGCAGCTTCCAGAAGAAGACC
787 MetLysLysGluAspPheAspIleTyrAspGluAspGluAsnGlnSerProArgSerPheGlnLysLysThr
PmlI
2449 CGCCACTACTTCATCGCCGCCGTGGAGCGCCTGTGGGACTACGGCATGAGCAGCAGCCCCCACGTGCTGCGC
811 ArgHisTyrPheIleAlaAlaValGluArgLeuTrpAspTyrGlyMetSerSerSerProHisValLeuArg
2521 AACCGCGCCCAGAGCGGCAGCGTGCCCCAGTTCAAGAAGGTGGTGTTCCAGGAGTTCACCGACGGCAGCTTC
835 AsnArgAlaGlnSerGlySerValProGlnPheLysLysValValPheGlnGluPheThrAspGlySerPhe
ApaI
2593 ACCCAGCCCCTGTACCGCGGCGAGCTGAACGAGCACCTGGGCCTGCTGGGCCCCTACATCCGCGCCGAGGTG
859 ThrGlnProLeuTyrArgGlyGluLeuAsnGluHisLeuGlyLeuLeuGlyProTyrIleArgAlaGluVal
BstEII
2665 GAGGACAACATCATGGTGACCTTCCGCAACCAGGCCAGCCGCCCCTACAGCTTCTACAGCAGCCTGATCAGC
883 GluAspAsnIleMetValThrPheArgAsnGlnAlaSerArgProTyrSerPheTyrSerSerLeuIleSer
2737 TACGAGGAGGACCAGCGCCAGGGCGCCGAGCCCCGCAAGAACTTCGTGAAGCCCAACGAGACCAAGACCTAC
907 TyrGluGluAspGlnArgGlnGlyAlaGluProArgLysAsnPheValLysProAsnGluThrLysThrTyr
2809 TTCTGGAAGGTGCAGCACCACATGGCCCCCACCAAGGACGAGTTCGACTGCAAGGCCTGGGCCTACTTCAGC
931 PheTrpLysValGlnHisHisMetAlaProThrLysAspGluPheAspCysLysAlaTrpAlaTyrPheSer

FIG. 9 (2 of 3)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955 AspValAspLeuGluLysAspValHisSerGlyLeuIleGlyProLeuLeuValCysHisThrAsnThrLeu
EagI
BstEII
2953 AACCCCGCCCACGGCCGCCAGGTGACCGTGCAGGAGTTCGCCCTGTTCTTCACCATCTTCGACGAGACCAAG
979 AsnProAlaHisGlyArgGlnValThrValGlnGluPheAlaLeuPhePheThrIlePheAspGluThrLys
3025 AGCTGGTACTTCACCGAGAACATGGAGCGCAACTGCCGCGCCCCCTGCAACATCCAGATGGAGGACCCCACC
1003 SerTrpTyrPheThrGluAsnMetGluArgAsnCysArgAlaProCysAsnIleGlnMetGluAspProThr
3097 TTCAAGGAGAACTACCGCTTCCACGCCATCAACGGCTACATCATGGACACCCTGCCCGGCCTGGTGATGGCC
1027 PheLysGluAsnTyrArgPheHisAlaIleAsnGlyTyrIleMetAspThrLeuProGlyLeuValMetAla
KpnI
3169 CAGGACCAGCGCATCCGCTGGTACCTGCTGAGCATGGGCAGCAACGAGAACATCCACAGCATCCACTTCAGC
1051 GlnAspGlnArgIleArgTrpTyrLeuLeuSerMetGlySerAsnGluAsnIleHisSerIleHisPheSer
PmlI
3241 GGCCACGTGTTCACCGTGCGCAAGAAGGAGGAGTACAAGATGGCCCTGTACAACCTGTACCCCGGCGTGTTC
1075 GlyHisValPheThrValArgLysLysGluGluTyrLysMetAlaLeuTyrAsnLeuTyrProGlyValPhe
3313 GAGACCGTGGAGATGCTGCCCAGCAAGGCCGGCATCTGGCGCGTGGAGTGCCTGATCGGCGAGCACCTGCAC
1099 GluThrValGluMetLeuProSerLysAlaGlyIleTrpArgValGluCysLeuIleGlyGluHisLeuHis
3385 GCCGGCATGAGCACCCTGTTCCTGGTGTACAGCAACAAGTGCCAGACCCCCCTGGGCATGGCCAGCGGCCAC
1123 AlaGlyMetSerThrLeuPheLeuValTyrSerAsnLysCysGlnThrProLeuGlyMetAlaSerGlyHis
ApaI
3457 ATCCGCGACTTCCAGATCACCGCCAGCGGCCAGTACGGCCAGTGGGCCCCCAAGCTGGCCCGCCTGCACTAC
1147 IleArgAspPheGlnIleThrAlaSerGlyGlnTyrGlyGlnTrpAlaProLysLeuAlaArgLeuHisTyr
3529 AGCGGCAGCATCAACGCCTGGAGCACCAAGGAGCCCTTCAGCTGGATCAAGGTGGACCTGCTGGCCCCCATG
1171 SerGlySerIleAsnAlaTrpSerThrLysGluProPheSerTrpIleLysValAspLeuLeuAlaProMet
3601 ATCATCCACGGCATCAAGACCCAGGGCGCCCGCCAGAAGTTCAGCAGCCTGTACATCAGCCAGTTCATCATC
1195 IleIleHisGlyIleLysThrGlnGlyAlaArgGlnLysPheSerSerLeuTyrIleSerGlnPheIleIle
3673 ATGTACAGCCTGGACGGCAAGAAGTGGCAGACCTACCGCGGCAACAGCACCGGCACCCTGATGGTGTTCTTC
1219 MetTyrSerLeuAspGlyLysLysTrpGlnThrTyrArgGlyAsnSerThrGlyThrLeuMetValPhePhe
(SmaI/EcoRV)
3745 GGCAACGTGGACAGCAGCGGCATCAAGCACAACATCTTCAACCCCCCCATCATCGCCCGCTACATCCGCCTG
1243 GlyAsnValAspSerSerGlyIleLysHisAsnIlePheAsnProProIleIleAlaArgTyrIleArgLeu
3817 CACCCCACCCACTACAGCATCCGCAGCACCCTGCGCATGGAGCTGATGGGCTGCGACCTGAACAGCTGCAGC
1267 HisProThrHisTyrSerIleArgSerThrLeuArgMetGluLeuMetGlyCysAspLeuAsnSerCysSer
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1291 MetProLeuGlyMetGluSerLysAlaIleSerAspAlaGlnIleThrAlaSerSerTyrPheThrAsnMet
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1315 PheAlaThrTrpSerProSerLysAlaArgLeuHisLeuGlnGlyArgSerAsnAlaTrpArgProGlnVal
BstEII
4033 AACAACCCCAAGGAGTGGCTGCAGGTGGACTTCCAGAAGACCATGAAGGTGACCGGCGTGACCACCCAGGGC
1339 AsnAsnProLysGluTrpLeuGlnValAspPheGlnLysThrMetLysValThrGlyValThrThrGlnGly
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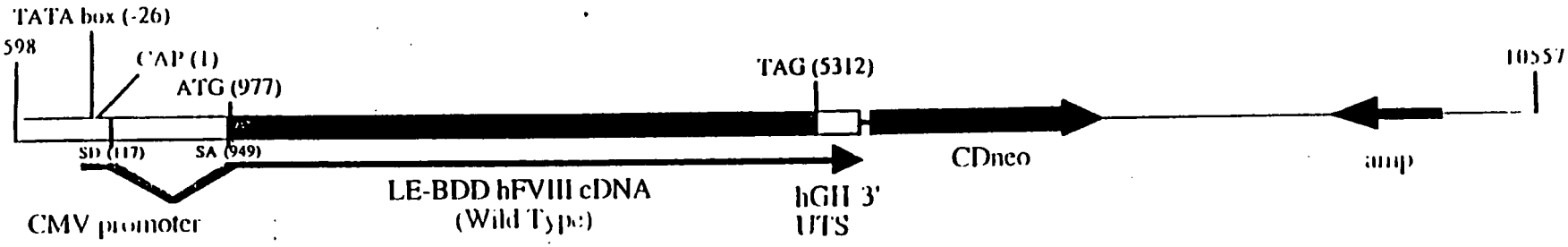
1363 ValLysSerLeuLeuThrSerMetTyrValLysGluPheLeuIleSerSerSerGlnAspGlyHisGlnTrp
4177 ACCCTGTTCTTCCAGAACGGCAAGGTGAAGGTGTTCCAGGGCAACCAGGACAGCTTCACCCCCGTGGTGAAC
1387 ThrLeuPhePheGlnAsnGlyLysValLysValPheGlnGlyAsnGlnAspSerPheThrProValValAsn
4249 AGCCTGGACCCCCCCCCTGCTGACCCGCTACCTGCGCATCCACCCCCAGAGCTGGGTGCACCAGATCGCCCTG
1411 SerLeuAspProProLeuLeuThrArgTyrLeuArgIleHisProGlnSerTrpValHisGlnIleAlaLeu
SmaI
HindIII
4321 CGCATGGAGGTGCTGGGCTGCGAGGCCCAGGACCTGTACTAGCTGCCCCGGGCTACAAGCTTTAC
1435 ArgMetGluValLeuGlyCysGluAlaGlnAspLeuTyr•••

FIG. 9 (3 of 3)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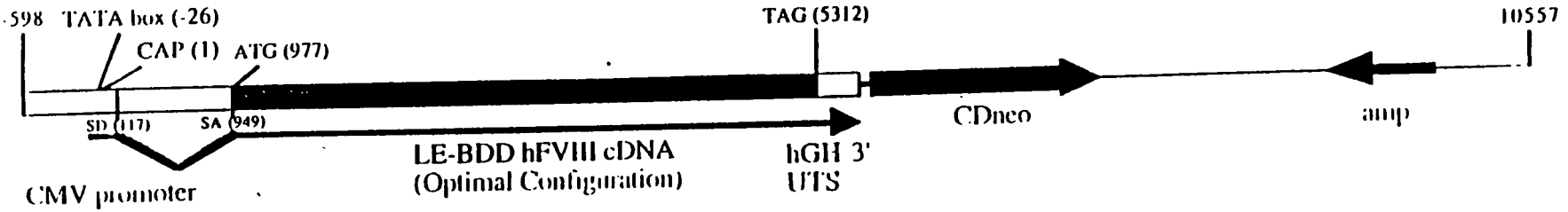
598
CAP (1)
ATG (977)
TAG (5312)
10557
SD (117)
SA (949)
CDneo
amp
LE-BDD hFVIII cDNA
(Wild Type)
hGH 3'
UTS
CMV promoter

FIG. 10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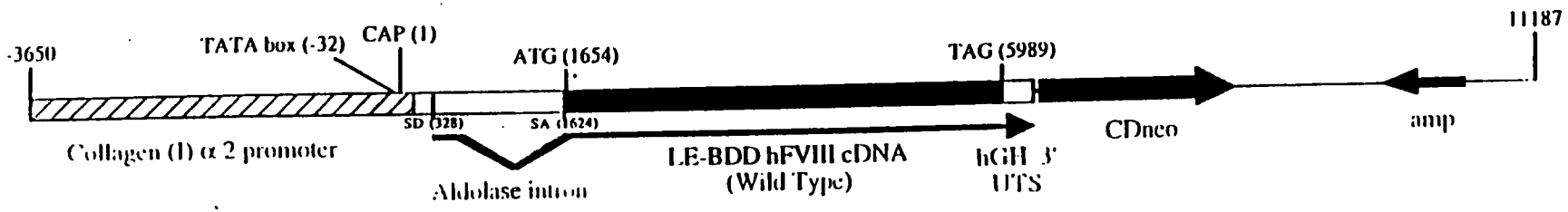
TATA box (-26)
CAP (1)
ATG (977)
TAG (5312)
10557
SD (117)
SA (949)
CDneo
amp
LE-BDD hFVIII cDNA
(Optimal Configuration)
hGH 3'
UTS
CMV promoter

FIG. 11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TATA box (-32)
CAP (1)
ATG (1654)
TAG (5989)
11187
SD (328)
SA (1624)
Collagen (I) α 2 promoter
Aldolase intron
LE-BDD hFVIII cDNA
(Wild Type)
hGH 3'
UTS
CDneo
amp

FIG. 12

SUBSTITUTE SHEET

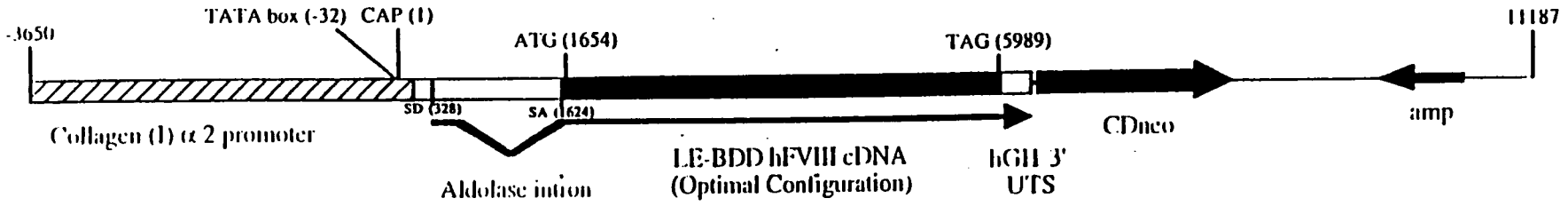

FIG. 13

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TKT
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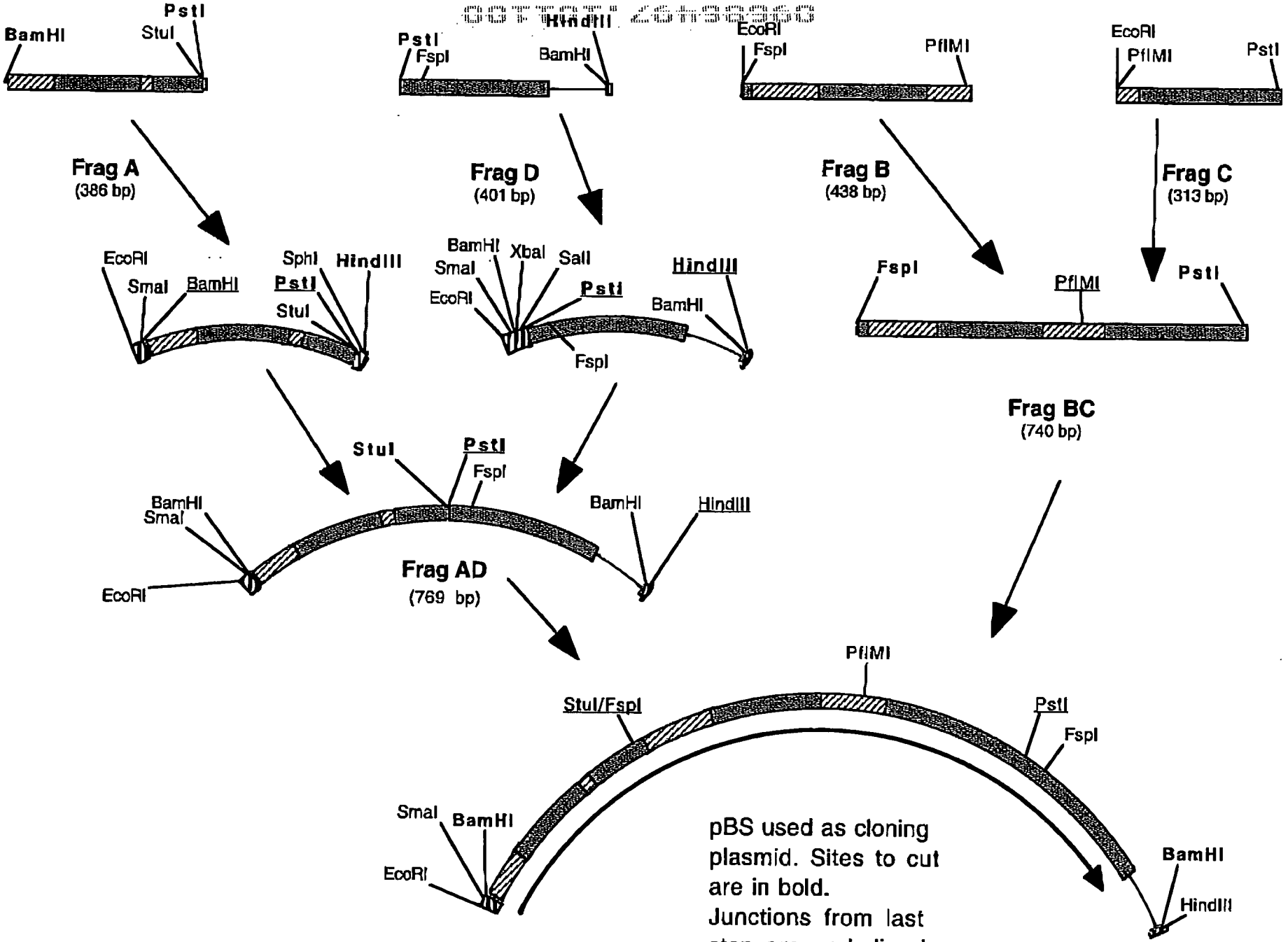

Fig. 14

GGATCCATGCAGCGCGTGAACATGATCATGGCCGAGAGCCCCGGCCTGATCACCATCTG
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GTTCGAGAACACCGAGCGCACCACCGAGTTCTGGAAGCAGTACGTGGACGGCGACCAGT
GCGAGAGCAACCCCTGCCTGAACGGCGGCAGCTGCAAGGACGACATCAACAGCTACGAG
TGCTGGTGCCCCTTCGGCTTCGAGGGCAAGAACTGCGAGCTGGACGTGACCTGCAACAT
CAAGAACGGCCGCTGCGAGCAGTTCTGCAAGAACAGCGCCGACAACAAGGTGGTGTGCA
GCTGCACCGAGGGCTACCGCCTGGCCGAGAACCAGAAGAGCTGCGAGCCCGCCGTGCCC
TTCCCCTGCGGCCGCGTGAGCGTGAGCCAGACCAGCAAGCTGACCCGCGCCGAGACCGT
GTTCCCCGACGTGGACTACGTGAACAGCACCGAGGCCGAGACCATCCTGGACAACATCA
CCCAGAGCACCCAGAGCTTCAACGACTTCACCCGCGTGGTGGGCGGCGAGGACGCCAAG
CCCGGCCAGTTCCCCTGGCAGGTGGTGCTGAACGGCAAGGTGGACGCCTTCTGCGGCGG
CAGCATCGTGAACGAGAAGTGGATCGTGACCGCCGCCCACTGCGTGGAGACCGGCGTGA
AGATCACCGTGGTGGCCGGCGAGCACAACATCGAGGAGACCGAGCACACCGAGCAGAAG
CGCAACGTGATCCGCATCATCCCCCACCACAACTACAACGCCGCCATCAACAAGTACAA
CCACGACATCGCCCTGCTGGAGCTGGACGAGCCCCTGGTGCTGAACAGCTACGTGACCC
CCATCTGCATCGCCGACAAGGAGTACACCAACATCTTCCTGAAGTTCGGCAGCGGCTAC
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TGAACTGGATCAAGGAGAAGACCAAGCTGACCTAATGAAAGATGGATTTCCAAGGTTAA
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Fig. 15

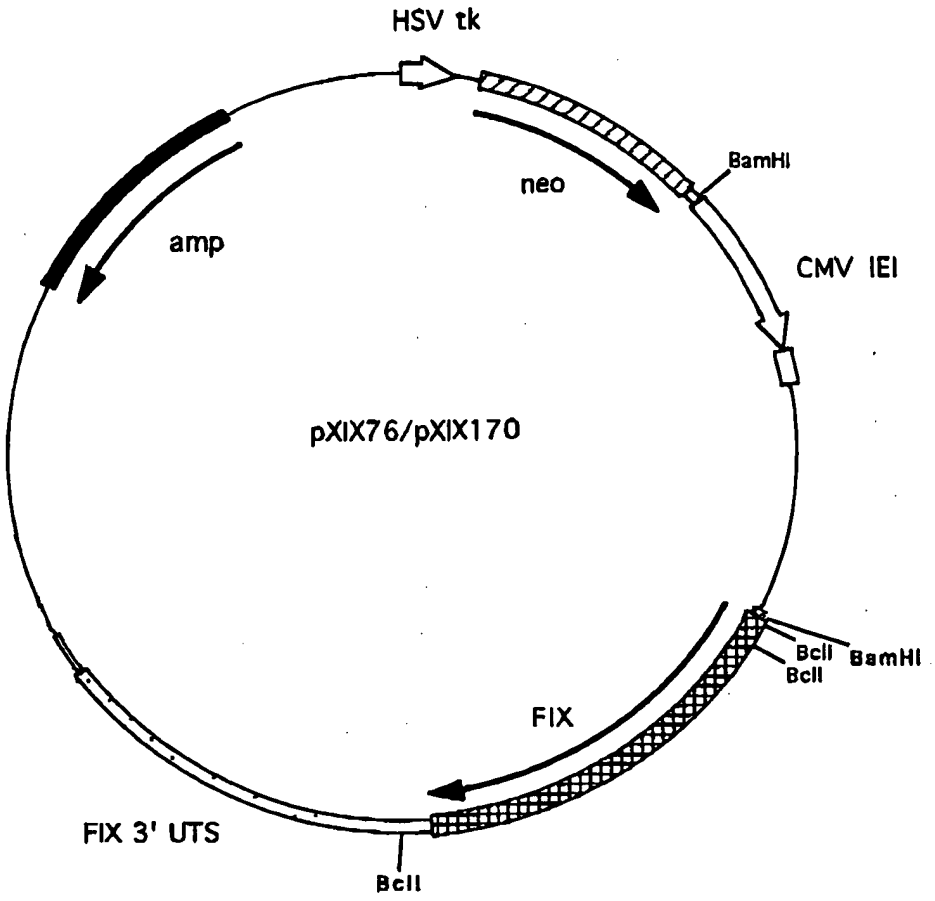
HSV tk
BamHI
neo
amp
CMV IEI
pXIX76/pXIX170
BclI
BamHI
BclI
FIX
FIX 3' UTS
BclI

Fig. 1c

GGATCCGCTAGAGCGGAAATTTATGCTGTCCGGTCACCGTGACAATGCAGCTGCGCAAC
CCCGAGCTGCACCTGGGCTGCGCCCTGGCCCTGCGCTTCCTGGCCCTGGTGAGCTGGGA
CATCCCCGGCGCCCGCGCCCTGGACAACGGCCTGGCCCGCACCCCCACCATGGGCTGGC
TGCACTGGGAGCGCTTCATGTGCAACCTGGACTGCCAGGAGGAGCCCGACAGCTGCATC
AGCGAGAAGCTGTTCATGGAGATGGCCGAGCTGATGGTGAGCGAGGGCTGGAAGGACGC
CGGCTACGAGTACCTGTGCATCGACGACTGCTGGATGGCCCCCCAGCGCGACAGCGAGG
GCCGCCTGCAGGCCGACCCCCAGCGCTTCCCCCACGGCATCCGCCAGCTGGCCAACTAC
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CGGCTTCCCCGGCAGCTTCGGCTACTACGACATCGACGCCCAGACCTTCGCCGACTGGG
GCGTGGACCTGCTGAAGTTCGACGGCTGCTACTGCGACAGCCTGGAGAACCTGGCCGAC
GGCTACAAGCACATGAGCCTGGCCCTGAACCGCACCGGCCGCAGCATCGTGTACAGCTG
CGAGTGGCCCCTGTACATGTGGCCCTTCCAGAAGCCCAACTACACCGAGATCCGCCAGT
ACTGCAACCACTGGCGCAACTTCGCCGACATCGACGACAGCTGGAAGAGCATCAAGAGC
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CTGGAACGACCCCGACATGCTGGTGATCGGCAACTTCGGCCTGAGCTGGAACCAGCAGG
TGACCCAGATGGCCCTGTGGGCCATCATGGCCGCCCCCCTGTTCATGAGCAACGACCTG
CGCCACATCAGCCCCCAGGCCAAGGCCCTGCTGCAGGACAAGGACGTGATCGCCATCAA
CCAGGACCCCCTGGGCAAGCAGGGCTACCAGCTGCGCCAGGGCGACAACTTCGAGGTGT
GGGAGCGCCCCCTGAGCGGCCTGGCCTGGGCCGTGGCCATGATCAACCGCCAGGAGATC
GGCGGCCCCCGCAGCTACACCATCGCCGTGGCCAGCCTGGGCAAGGGCGTGGCCTGCAA
CCCCGCCTGCTTCATCACCCAGCTGCTGCCCGTGAAGCGCAAGCTGGGCTTCTACGAGT
GGACCAGCCGCCTGCGCAGCCACATCAACCCCACCGGCACCGTGCTGCTGCAGCTGGAG
AACACCATGCAGATGAGCCTGAAGGACCTGCTGTAAAAAAAAAAAAAACTCGAG

09 09 09 09 09 09 09 09 09 09

Fig. 17

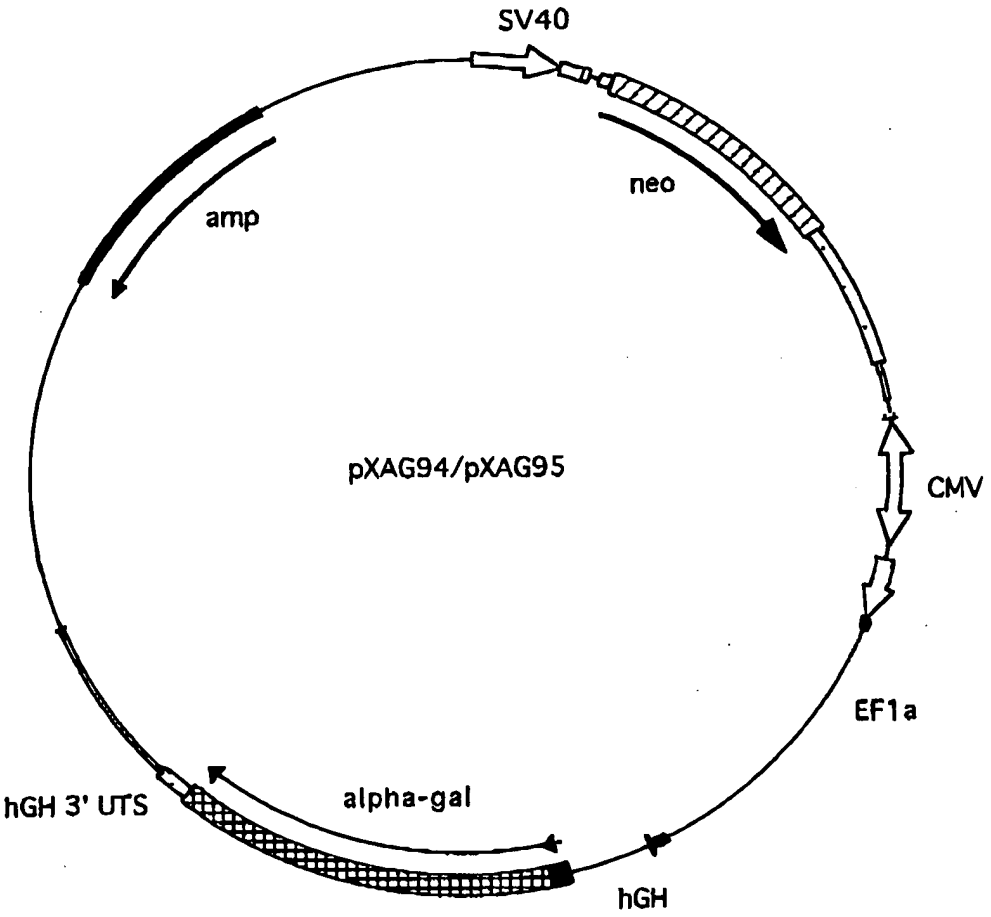

Fig 18

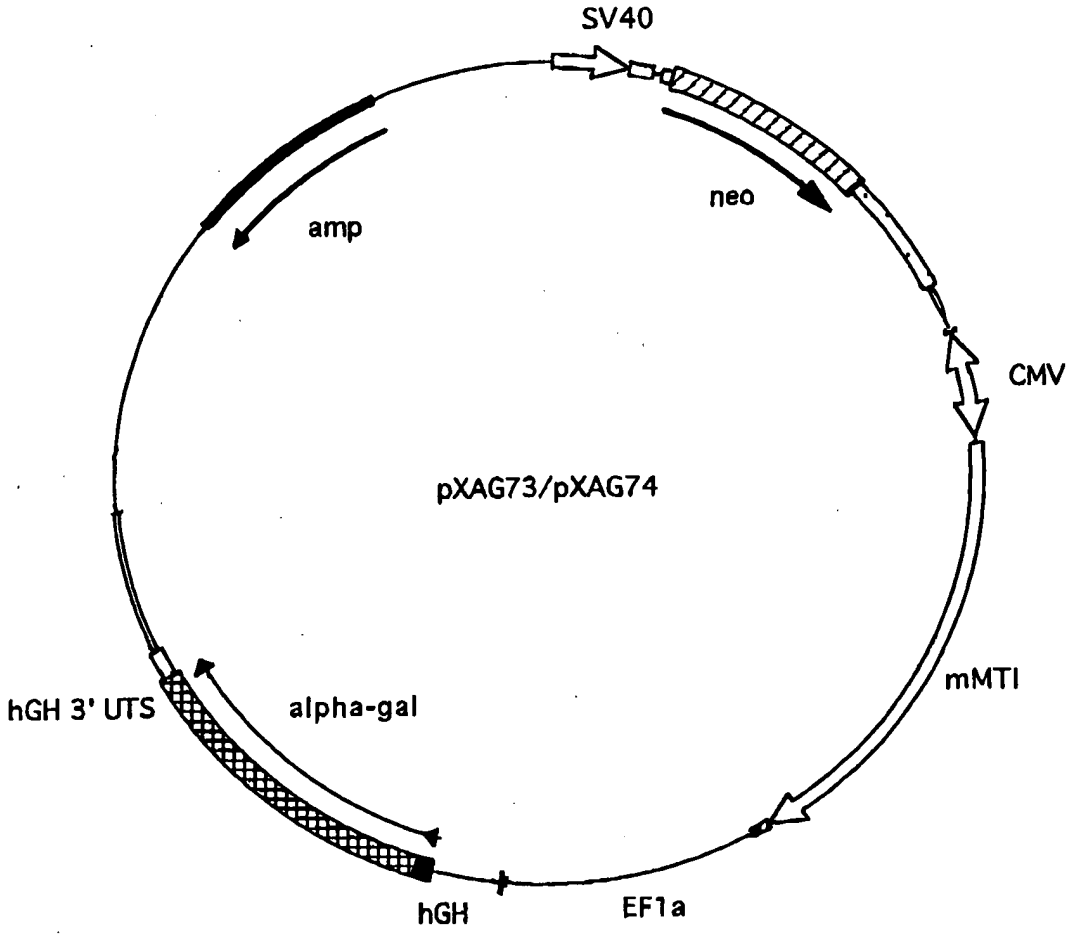
SV40
neo
amp
CMV
pXAG73/pXAG74
mMTI
hGH 3' UTS
alpha-gal
hGH
EF1a

Fig. 19